



Food and Drug Administration  
9200 Corporate Boulevard  
Rockville MD 20850

Mr. Gregory P. Payne, R. A. C.  
Principal Regulatory Specialist  
Hybritech, Inc.  
8958 Terman Court  
San Diego, California 92196-9006

MAR 10 1998

Re: P970038

Tandem®-R free PSA assay and Tandem®-MP free PSA assay

Filed: August 29, 1997

Amended: December 19, 1997; January 7, 1998; February 23,  
and March 5, 1998

Dear Mr. Payne:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Tandem®-R free PSA assay and Tandem®-MP free PSA assay. The Tandem®-R free PSA Immunoradiometric Assay is an In Vitro device for the quantitative measurement of free prostate specific antigen (free PSA) in human serum. The Tandem®-MP free PSA Immunoenzymetric Assay is an In Vitro device for the quantitative measurement of free prostate specific antigen (free PSA) in human serum. Hybritech's Tandem free PSA assays are intended to be used with Tandem (total) PSA to calculate the ratio of free PSA to total PSA expressed as a percentage (percent free PSA). Percent free PSA as measured by Hybritech's Tandem assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection in men aged 50 years and older with total PSA between 4 and 10 ng/mL and digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

Expiration dating for this device has been established and approved at 10 weeks when stored between 2°C and 8°C for the Tandem-R free PSA assay and at 6 months when stored between 2°C and 8°C for the Tandem-MP free PSA assay.

CDRH will notify the public of its decision to approve your PMA by making available a summary of the safety and effectiveness data upon which the approval is based. The information can be found on the FDA CDRH Internet HomePage located at <http://www.fda.gov/cdrh/pmapage.html>. Written requests for this information can also be made to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr.,

rm. 1-23, Rockville, MD 20857. The written request should include the PMA number or docket number. Within 30 days from the date that this information is placed on the Internet, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)  
Center for Devices and Radiological Health  
Food and Drug Administration  
9200 Corporate Blvd.  
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Dr. Peter Maxim at (301) 594-1293.

Sincerely yours,

*Kimber C. Richter*

Kimber C. Richter, M.D.  
Deputy Director for Clinical and Review Policy  
Office of Device Evaluation  
Center for Devices and Radiological Health

Enclosure

#### CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mix-up of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984. This regulation was replaced by the reporting requirements of the Safe Medical Devices Act of 1990 which became effective July 31, 1996 and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to the FDA whenever they receive or otherwise become aware of information, from any source, that reasonably suggests that a device marketed by the manufacturer or importer:

- (1) May have caused or contributed to a death or serious injury; or
- (2) Has malfunctioned and such device or similar device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for a PMA, the manufacturer shall submit the appropriate reports required by the MDR Regulation within the time frames as identified in 21 CFR 803.10(c) using FDA Form 3500A, i.e., 30 days after becoming aware of a reportable death, serious injury, or malfunction as described in 21 CFR 803.50 and 21 CFR 803.52 and 5 days after becoming aware that a reportable MDR event requires remedial action to prevent an unreasonable risk of substantial harm to the public health. The manufacturer is responsible for submitting a baseline report on FDA Form 3417 for a device when the device model is first reported under 21 CFR 803.50. This baseline report is to include the PMA reference number. Any written report and its envelope is to be specifically identified, e.g., "Manufacturer Report," "5-Day Report," "Baseline Report," etc. Any written report is to be submitted to:

Food and Drug Administration  
Center for Devices and Radiological Health  
Medical Device Reporting  
PO Box 3002  
Rockville, Maryland 20847-3002

Copies of the MDR Regulation (FOD # 336&1336) and FDA publications entitled "An Overview of the Medical Device Reporting Regulation" (FOD # 509) and "Medical Device Reporting for Manufacturers" (FOD #987) are available on the CDRH WWW Home Page. They are also available through CDRH's Fact-On-Demand (F-O-D) at

800-899-0381. Written requests for information can be made by sending a facsimile to CDRH's Division of Small Manufacturers Assistance (DSMA) at 301-443-8818.

# COMPREHENSIVE SUMMARY OF SAFETY AND EFFECTIVENESS FOR TANDEM free PSA

## ***I. General Information***

### **Generic Name**

Free Prostate Specific Antigen (free PSA), Immunological  
Test System for the Detection of Prostate Cancer

### **Trade Names**

Tandem®-R free PSA ImmunoRadioMetric Assay

Tandem®-MP free PSA ImmunoEnzyMetric Assay

### **Applicant's Name and Address**

Hybritech Incorporated  
8958 Terman Court  
San Diego, California 92121

Premarket Approval Application (PMA) Number P970038

Date of Panel Recommendation February 2, 1998

Date of Notice of Approval to the Applicant MAR 10 1998

## ***II. Indications for Use***

The Tandem®-R free PSA Immunoradiometric Assay is an *In Vitro* device for the quantitative measurement of free prostate specific antigen (free PSA) in human serum. The Tandem®-MP free PSA Immunoenzymetric Assay is an *In Vitro* device for the quantitative measurement of free prostate specific antigen (free PSA) in human serum.

Hybritech's Tandem free PSA assays are intended to be used with Tandem (total) PSA to calculate the ratio of free PSA to total PSA expressed as a percentage (percent free PSA).

Percent free PSA as measured by Hybritech's Tandem assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection in men aged 50 years and older with total PSA between 4 and 10 ng/mL and digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer.

## Background

Prostate cancer is the most common type of cancer found in men in the United States, with an incidence of approximately one case for every ten men. It is also the second leading cause of cancer deaths among American men.<sup>1</sup>

The American Medical Association and the American Urological Association have recommended that all men 50 years of age and older have an annual prostate evaluation consisting of a digital rectal examination (DRE) and a serum total prostate specific antigen (PSA) test<sup>1</sup>. Performed together, DRE and PSA detect approximately 81% more prostate cancers than DRE alone.<sup>2</sup>

However, the incidence of false positive results, that is, men who have a positive PSA result in the range of 4 to 10 ng/mL but are histologically negative for cancer at biopsy, is approximately 75%.<sup>2</sup> Therefore, an adjunct test which aids in the discrimination of prostate cancer and BPH would be beneficial.

PSA was identified and purified by Wang and co-workers in 1979.<sup>3</sup> PSA, a serine protease, is produced by the epithelial cells of the prostate and is produced by both benign and malignant cells. Abnormalities in the prostate gland architecture resulting from trauma or disease can lead to "leakage" of PSA into the bloodstream. PSA exists primarily as three forms in human serum.<sup>4</sup> One form of PSA is believed to be enveloped by alpha-2-macroglobulin, and has been shown to lack immunoreactivity.<sup>5</sup> A second form of circulating PSA is complexed to another protease inhibitor, alpha-1-antichymotrypsin (ACT).<sup>5,6</sup> The third form of PSA is not complexed to protease inhibitor, and is termed free PSA.<sup>5,6</sup> The latter two forms are immunologically detectable in commercially available assays and are referred to as total PSA.

It has been demonstrated that measurement of PSA forms is useful in differentiation of prostate cancer from benign prostatic conditions.<sup>7,8</sup> In patients with elevated PSA concentrations, men with prostate cancer tend to have lower percent free PSA (free PSA/total PSA) values than do men with benign disease.<sup>9-12</sup> This difference in the distribution of percent free PSA (%fPSA) values in men with and without cancer may be recommended to select cutoffs for a biopsy decision, maintaining 90% to 95% sensitivity, while sparing 20% to 30% of men with benign disease from biopsy. Alternatively, it may be recommended for risk assessment, to determine the probability of cancer for an individual patient. Lower %fPSA values are associated with higher risk of cancer.<sup>9-12</sup>

Studies have shown that %fPSA cutoffs and clinical performance differ when various combinations of free and total PSA assays from different manufacturers are used.<sup>13-16</sup> Mean %fPSA values from identical serum samples may be two-fold higher using different assay combinations,<sup>14</sup> and 95% sensitivity cutoffs may vary from 22% to 34% using different assay combinations.<sup>16</sup>

### **III. Device Descriptions**

The Tandem-R® free PSA assay is a solid-phase, two-site immunoradiometric assay employing two murine monoclonal antibodies directed toward two distinct epitopes on the free PSA antigen. Serum samples containing free PSA are incubated with a plastic bead (solid phase) coated with monoclonal antibody directed toward a specific site on the free PSA molecule and with a radiolabeled monoclonal antibody directed toward a second site on the free PSA molecule. Following the formation of the solid-phase/free PSA/labeled antibody sandwich, the bead is washed to remove unbound labeled antibody. The radioactivity bound to the solid phase is measured in a gamma counter and is directly proportional to the amount of free PSA contained in the test sample. The amount of free PSA in the test sample is determined from a standard curve of the measured radioactivity and free PSA Calibrators containing from 0 to 20 ng free PSA/mL concurrently tested with test samples.

The Tandem®-MP free PSA assay is a solid-phase, two-site immunoenzymetric assay. Samples containing free PSA are incubated with a solution containing two treated free PSA-specific monoclonal antibodies. One is enzyme-labeled; the other is biotin-labeled. The reaction takes place in a plastic microplate (solid phase) consisting of a frame and several strips of wells coated with streptavidin (which binds with biotin). Following the formation of a solid phase/capture antibody/free PSA/labeled antibody sandwich, the microplate is washed to remove unbound labeled antibody and is then incubated with an enzyme substrate. The amount of substrate turnover is determined colorimetrically by measuring the absorbance of the quenched reaction at 450 nm. The absorbance is proportional to the concentration of free PSA present in the test sample. The calculation of free PSA concentration in the test sample is determined from a standard curve of the measured absorbance and free PSA Calibrators containing from 0 to 20 ng free PSA/mL concurrently tested with test samples.

### **WARNINGS, PRECAUTIONS, CONTRAINDICATIONS**

Study results were obtained using Hybritech's Tandem® free PSA and total PSA assays. Studies have shown that %fPSA cutoffs

and clinical performance differ when various combinations of free and total PSA assays from different manufacturers are used.<sup>13-16</sup> Mean %fPSA values from identical serum samples may be two-fold higher using different assay combinations,<sup>14</sup> and 95% sensitivity cutoffs may vary from 22% to 34% using different assay combinations.<sup>16</sup>

Use of a total PSA assay not approved for cancer detection or use of one manufacturer's free PSA assay with another manufacturer's total PSA assay may result in:

- 1) an inappropriate population of patients selected for follow-up percent free PSA testing; and
- 2) significantly different percent free PSA values, cutoffs and cancer probabilities than those presented in the Expected Values section of this insert.

Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by or on the order of a physician.

There are no known contraindications for the Tandem-R and Tandem-MP free PSA assays

#### ***IV. Alternative Practices and Procedures***

A patient with a positive PSA (>4.0 ng PSA/mL) and/or a suspicious digital rectal examination is typically recommended for prostate biopsy. Biopsy is the definitive method of diagnosis. Transrectal ultrasonography of the prostate (TRUS) may provide the clinician with additional information about prostatic abnormalities. However, TRUS is usually not performed as a stand-alone diagnostic procedure. Rather, it is typically used to direct the biopsy to suspicious areas of the gland.

The concepts of PSA density, PSA velocity, and age-specific reference ranges for PSA have met with minimal success in assisting physicians in patient management.

#### ***V. Marketing History***

Tandem®-R free PSA has been marketed in the following countries since its introduction in August 1995:

Belgium	Spain
Luxembourg	The United Kingdom
Netherlands	France
Germany	Austria
Italy	Switzerland

Sweden  
Greece

South Africa  
The Czech Republic.

There have been no reports of adverse events connected to the use of Tandem-R® free PSA in any country since its introduction. The device has not been withdrawn from the market in any country for reasons relating to its safety or effectiveness.

## ***VI. Potential Adverse Effects of the Device on Health***

Some patients with prostate cancer may have %fPSA values that exceed the recommended cutoff (false negatives). A patient could be adversely affected by a delay in the treatment of the malignancy.

Value of %fPSA less than the recommended cutoff may be observed in patients with non-malignant conditions of the prostate or in non-prostate malignancies (false positives).

## ***VII. Summary of Studies***

### **A. Pre-clinical Studies**

Pre-clinical studies were conducted at Hybritech Incorporated to determine the purity and specificity of the reagents, as well as the performance characteristics of the assays.

#### **1. Characterization of the Antigen**

The free PSA antigen is identical to one for which there is an approved PMA. The PSA used in the immunization of mice to create the monoclonal antibodies found in the Tandem®-R total PSA, Tandem®-R free PSA, and Tandem®-MP free PSA assays was purified from human prostate tissue. The PSA used to prepare the Primary Reference Preparation and Stock Standard (used to prepare the Calibrators and Controls) for the Tandem®-R total PSA, Tandem®-R free PSA, and Tandem®-MP free PSA assays was isolated and purified from human seminal fluid.

The biochemical and immunochemical properties of the PSA from prostate tissue and the PSA from seminal fluid are known to be identical.

#### **2. Specificity of the Antibodies**

The solid-phase antibody is the same antibody for a device for which there is an approved PMA. This

antibody was shown, using immunohistochemical methods, to bind specifically to human seminal fluid PSA.

The radio- and enzyme-labeled (reporter) antibody is unique to the Tandem-R® and Tandem®-MP free PSA assays. This antibody was shown by immunochemical methods to be specific for the free PSA molecule.

### 3. Performance Characteristics

#### (a) Reproducibility

Within-run, between-run and total reproducibility of the Tandem free PSA assays were determined at Hybritech Incorporated. The testing protocol was based on NCCLS Guideline EP5-T2 (Evaluation of Precision Performance of Clinical Chemistry Devices).

In these experiments, five samples containing free PSA concentrations spanning the assay range were assayed in duplicate. Three kit lots were used in the testing. The results are presented in Tables 1 and 2. The percent coefficients of variation ranged from 1.08 to 6.76 for Tandem-R® free PSA and 1.85 to 7.89 for Tandem-MP free PSA, respectively.

Table 1  
Tandem®-R free PSA  
Assay Reproducibility

Sample n = 40	Mean (ng FPSA/mL)	Within-run		Between-run		Total	
		S.D.	%CV	S.D.	%CV	S.D.	%CV
1	0.42	0.017	4.02	0.023	5.44	0.029	6.76
2	1.03	0.034	3.29	0.035	3.39	0.049	4.73
3	2.00	0.037	1.85	0.073	3.64	0.082	4.09
4	4.08	0.044	1.08	0.117	2.87	0.125	3.06
5	15.47	0.292	1.89	0.175	1.13	0.340	2.20

Table 2  
Tandem®-MP free PSA  
Assay Reproducibility

Sample n = 40	Mean (ng FPSA/mL)	Within-run		Between-run		Total	
		S.D.	%CV	S.D.	%CV	S.D.	%CV
1	0.40	0.014	3.53	0.028	7.05	0.031	7.89
2	1.02	0.040	3.92	0.028	2.75	0.049	4.79
3	1.89	0.035	1.85	0.082	4.34	0.089	4.72
4	3.50	0.124	3.54	0.144	4.11	0.190	5.43
5	15.72	0.616	3.92	0.465	2.96	0.772	4.91

The between-laboratory reproducibility of the Tandem free PSA assay was evaluated in three separate laboratories at Hybritech Incorporated. Five samples with free PSA values spanning the range of 0 to 20 ng free PSA/mL were tested. Each sample was run in triplicate in a total of nine assays per site using three kit lots. The mean, standard deviation and percent coefficient of variation (%CV) were determined for each sample. These results are presented in Tables 3 and 4.

For the Tandem®-R free PSA assay, the between-laboratory variability observed across the concentrations tested ranged from 0.55% to 3.13%.

For the Tandem®-MP free PSA assay, the between-laboratory variability observed across the concentrations tested ranged from 0.55% to 3.31%. These results are within the acceptable limits for devices of this type.

**Table 3**  
**Tandem®-R free PSA**  
**Reproducibility Between Laboratories**

	Mean (ng free PSA/mL)				
Sample n = 9	Site 1	Site 2	Site 3	Overall mean	%CV Between Sites
1	0.31	0.33	0.32	0.32	3.13
2	1.04	1.05	1.05	1.05	0.55
3	2.02	2.07	2.06	2.05	1.29
4	6.09	6.32	6.32	6.24	2.13
5	15.53	15.68	15.69	15.63	0.57

**Table 4**  
**Tandem®-MP free PSA**  
**Reproducibility Between Laboratories**

	Mean (ng free PSA/mL)				
Sample n = 9	Site 1	Site 2	Site 3	Overall mean	%CV Between Sites
1	0.40	0.39	0.39	0.39	1.47
2	1.04	1.05	1.04	1.04	0.55
3	1.90	1.87	1.88	1.88	0.81
4	3.63	3.40	3.55	3.53	3.31
5	16.00	15.50	15.80	15.80	1.60

(b) Sensitivity

1. Tandem®-R free PSA

The analytical sensitivity, or minimum detectable concentration (MDC), of the Tandem®-R free PSA assay was defined as that concentration of free PSA that corresponded to the counts per minute (cpm) that were two standard deviations greater than the mean cpm of 21 replicate determinations of the Zero Diluent/Calibrator.

The MDC was determined by assaying the Zero Diluent/Calibrator in replicates of 21 using three different reagent combinations for a total of 27 assays. The mean value of all the calculated MDC (from the 27 separate assays) was 0.02 ng free PSA/mL.

## 2. Tandem®-MP free PSA

The analytical sensitivity, or minimum detectable concentration (MDC), of the Tandem®-MP free PSA assay was defined as that concentration of free PSA that corresponded to the absorbance that was two standard deviations greater than the mean absorbance of 22 replicate determinations of the Zero Diluent/Calibrator.

The MDC was determined by assaying the Zero Diluent/Calibrator in replicates of 22 using nine different reagent combinations over three days for a total of 27 assays. The mean value of all the calculated MDC (from the 27 separate assays) was 0.02 ng free PSA/mL.

### (c) Specimen Dilution

Twelve serum specimens containing elevated concentrations of free PSA were diluted into the Zero Diluent/Calibrator to yield several concentrations. Each dilution study was analyzed by means of linear regression, yielding correlation coefficients that ranged from 0.9972 to 0.9999 and 0.9962 to 1.000 for the Tandem®-R free PSA assay and the Tandem®-MP free PSA assay, respectively.

### (d) Specificity

The analytical specificity of the Tandem free PSA assays was evaluated at Hybritech, Inc. by testing three patient serum pools. The pools were split and tested both unspiked (control) and spiked (test) with high levels of various potential interfering substances. Testing was performed according to NCCLS Guideline EP7-P (Interference Testing in Clinical Chemistry). Each control and test sample were assayed in quadruplicate. The substances tested and their concentrations are listed in Table 5.

Table 5  
Tandem free PSA  
Interfering Substances Tested

Substance	Concentration
triglycerides	2500 mg/dL
total protein (Tandem <sup>®</sup> -R)	4 and 13 g/dL
total protein (Tandem <sup>®</sup> -MP)	4 and 17 g/dL
hemoglobin	500 mg/dL
bilirubin, unconjugated	20 mg/dL
bilirubin, conjugated	20 mg/dL
acetaminophen	0.2 mg/mL
aspirin	0.5 mg/mL
ibuprofen	0.4 mg/mL
naproxin sodium (Naprosen)	1.0 mg/mL
hydrocodone (Vicodin)	240 ng/mL
cimetidine (Tagamet)	0.1 mg/mL
capotril (Capoten)	4.0 mg/mL
lovastatin (Mevacor)	270 ng/mL
nifedipine (Procardia)	270 ng/mL
metoprolol (Lopressor)	2.7 mg/mL
finasteride	370 ng/mL
terazosin hydrochloride	1.45 mg/mL
ciprofloxacin	46 mg/mL
* sulfamethoxazole	116.5 mg/mL
* trimethoprim	9.7 mg/mL
doxycycline	2.6 mg/mL
clomipramine HCl (Anafril)	2.7 mg/mL
fluoxetine (Prozac)	0.55 mg/mL
furosemide (Lasix)	20 mg/mL
methotrexate	13.2 mg/mL
prednisone	1.65 mg/mL
tavist-1	2.7 mg/mL

\* These two drugs tested in combination.

The results of the interfering substances analysis demonstrated that there was no significant interference effect from any of the substances tested.

The cross reactivity of PSA-ACT in the free PSA assay has been determined to be less than 1%.

(e) Stability

1) Tandem<sup>®</sup>-R free PSA

Three lots of Tandem<sup>®</sup>-R free PSA components (Beads, Tracer and Calibrators) were stored between 2°C and 8°C and tested at specified intervals at Hybritech Incorporated. The results of this testing supported expiration dating of at least 12 months for the beads and calibrators. The expiration dating of the Tandem<sup>®</sup>-R free PSA kit was

defined as the expiration dating of the tracer component. Since the tracer antibody had a stability of 10 weeks, the expiration dating for the Tandem®-R free PSA kit has been established at 10 weeks for product stored between 2°C and 8°C as indicated in the product labeling.

## 2) Tandem®-MP free PSA

Three lots of Tandem®-MP free PSA components (Conjugate and Calibrators) were stored between 2°C and 8°C and tested at specified intervals at Hybritech Incorporated. The results of this testing supported expiration dating of at least 6 months for the calibrators. The expiration dating of the Tandem®-MP free PSA kit is defined as the expiration date of the antibody conjugate component. Since the conjugate antibody has a stability of 6 months, the expiration dating for the Tandem-MP free PSA kit has been established at 6 months for product stored between 2°C and 8°C as indicated in the product labeling.

## B. Clinical Study

A multicenter, prospective clinical trial was conducted to evaluate the safety and effectiveness of %fPSA as measured by Hybritech's Tandem Assays (Tandem free PSA / Tandem total PSA x 100) as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection in men 50 years of age and older with total PSA between 4 and 10 ng/mL and digital rectal examination (DRE) findings that are not suspicious for cancer.

### 1. Study Sites

The study was performed at seven university medical centers nationwide. The principal investigators and their institutions are shown in Table 6.

Table 6

## Study Sites and Principal Investigators

Institution, City, State	Principal Investigators
Washington University St. Louis Missouri	William J. Catalona, MD - Chief, Urologic Surgery Timothy L. Ratliff, PhD - Director, Urologic Research
University of California Los Angeles, California	Jean B. deKernion, MD - Chief, Division of Urology
Brigham and Women's Hospital (Harvard) Boston, Massachusetts	Jerome P. Richie, MD - Chairman, Dept. of Urology Milenko J. Tanasijevic, MD - Medical Dir., Clin. Chem.
Loyola University Chicago, Illinois	Robert C. Flanigan, MD - Chairman, Dept. of Urology Robert E. Henkin, MD - Director of Nuclear Medicine
Johns Hopkins Hospital Baltimore, Maryland	Patrick C. Walsh, MD - Chairman, Dept. of Urology Alan W. Partin, MD - Assoc. Prof., Dept. of Urology Daniel W. Chan, PhD - Director, Clinical Chemistry
Baylor College of Medicine Houston, Texas	Peter T. Scardino, MD - Chairman, Dept. of Urology Kevin M. Slawin, MD - Asst. Professor, Dept. of Urology Dolores J. Lamb, PhD - Laboratory Director
University of Washington Seattle, Washington	Paul H. Lange, MD - Chairman, Dept. of Urology Michael K. Brawer, MD - Chief, Urology, Seattle VA Mark H. Wener, MD - Director of Immunology

## 2. Study Objective

The clinical trial study objective was to determine if %fPSA could spare men with benign disease from biopsy, with minimal loss in cancer detection. To evaluate this, Receiver Operator Characteristic (ROC) curves were generated for %fPSA and for total PSA, and the %fPSA cutoff was determined which detected 95% of the cancers (sensitivity). The corresponding percent decrease in biopsies in men with benign disease (specificity) was then determined for this cutoff. Area under the ROC curve (AUC) was compared for %fPSA and total PSA.

## 3. Subject Selection and Exclusion Criteria, Study Population, Study Period

Subjects were men 50 to 75 years of age who had not been treated for prostate disease at the time of the blood sampling. All men had total PSA concentrations between 4.0 and 10.0 ng/mL, a digital rectal examination (DRE) that was not suspicious for cancer,

and all underwent ultrasound-guided needle biopsy of the prostate (i.e., all men had a histologically-confirmed diagnosis). Subjects with benign disease were required to have undergone a six-core sextant biopsy in order to exclude the possibility of cancer. Exclusion criteria included acute prostatitis, urinary tract infection, prior transurethral resection of the prostate (TURP), or recent manipulation or medication that might alter serum total PSA concentrations. Serum samples were obtained from men meeting study entry criteria at seven university medical centers between July 1994 and December 1996. A total of 773 subjects (379 prostate cancer, 394 benign) were enrolled in the study. Racial distribution was as follows: 86% Caucasian, 9% African-American, 3% Hispanic, and 2% Asian.

#### 4. Study Population Demographics

Demographic and clinical characteristics were comparable for benign and cancer subject groups at study entry median age = 64 and 64 years respectively median total PSA = 5.6 and 5.9 ng/mL respectively. Median %fPSA values were statistically significantly lower in the cancer group (12% fPSA) than in the benign group (18% fPSA) ( $p = 0.0001$ ).

#### 5. Safety and Effectiveness Data

Study results demonstrated that %fPSA enhanced the specificity of testing with total PSA for prostate cancer detection. If a cutoff of  $\leq 25\%$  fPSA were used (i.e., only men with less than or equal to this cutoff had been biopsied), 95% (95% C.I.: 92-97%) of the cancers would have been detected (sensitivity) and 20% (95% C.I.: 16-24%) of the men with benign disease would have been spared biopsy (specificity) (Table 7). Since 75% of men with total PSA between 4 and 10 ng/mL and benign rectal examination findings had negative biopsy results, this represented a number of men who would not be required to undergo a biopsy.

Table 7

## Calculations and Sample Size for %fPSA ROC Curve

%fPSA Cutoffs	Sensitivity (# of cancers detected/ # of total cancers)			Specificity (# of non-cancers detected/ # of total non-cancers)		
	%	(n/N)	95% CI	%	(n/N)	95% CI
≤ 0 %	0%	(0/379)		100%	(394/394)	
≤ 5 %	6%	(24/379)	4 - 9%	99%	(391/394)	97-100%
≤ 10 %	36%	(136/379)	31-41%	91%	(357/394)	88-94%
≤ 15 %	64%	(241/379)	59-69%	68%	(266/394)	63-73%
≤ 20 %	84%	(318/379)	80-87%	40%	(156/394)	35-45%
≤ 22 %	90%	(341/379)	86-93%	29%	(119/394)	25-34%
≤ 25 %	95%	(358/379)	92-97%	20%	(80/394)	16-24%
≤ 32 %	98%	(373/379)	96-99%	6%	(25/394)	4-9%
≤ 55 %	100%	(379/379)		0%	(0/394)	

Shaded area = Primary study endpoint (%fPSA cutoffs which yielded sensitivities of 98%, 95%, and 90%, and the corresponding specificities [decrease in unnecessary biopsies] associated with each cutoff.)

The AUC was statistically significantly greater for %fPSA (0.72) than for total PSA (0.53) ( $p = 0.0001$ ), confirming that %fPSA was more predictive than PSA in this cohort of men with PSA between 4 and 10 ng/mL and a non-suspicious DRE.

The %fPSA values decreased as total PSA increased ( $r = -0.14$ ,  $p = 0.0001$ ). This relationship was not sufficiently robust to affect the cutoff in a population with total PSA of 4.0 to 10.0 ng/mL. No data was available to assess the affect of total PSA and %fPSA outside of the 4 - 10 ng/mL range. The %fPSA values increased as patient age increased ( $r = 0.34$ ,  $p = 0.0001$ ). Because of this relationship, age-specific cutoffs could result in more undetected cancers (lower sensitivity) in younger men. In contrast, a single 25% fPSA cutoff across all age groups resulted in the highest sensitivity (98%) in the younger men. In younger men whom are most likely to gain from early detection, use of a single 25% fPSA cutoff could result in a higher sensitivity (98%) and are more likely to benefit from a recommendation for biopsy.

The %fPSA values increased as prostate volume increased ( $r = 0.55$ ,  $p = 0.0001$ ). The cancers occurring in men with %fPSA values above the 25%

cutoff which would be missed were found primarily in older men with larger glands. These cancers also had pathologic features associated with indolent or latent, non-aggressive cancers, which develop slowly and would not be expected to affect the patient's lifespan. As tumor grade and tumor volume increased, %fPSA decreased. Only 7% of the cancer patients with %fPSA above the cutoff (versus 30% of those below the cutoff) had advanced tumor grade (Gleason scores  $\leq 7$ ).

## 6. Conclusions Drawn From Studies

The clinical trial demonstrated that the use of %fPSA as measured by the Tandem assays could increase the specificity of Tandem (total) PSA for prostate cancer detection in men with PSA levels of 4.0 to 10.0 ng/mL and benign findings on DRE. The resulting clinical benefit was a 20% reduction in unnecessary biopsies in men undergoing evaluation for prostate cancer with a minimal loss in overall cancer detection. The Tandem free and (total) PSA assays could provide physicians with a tool to assess the risk of cancer in their patients and to select the most appropriate follow-up measures for that patient.

The finding is also clinically advantageous to older men with prostate cancer (those with less than a 10 year life expectancy) who are often not affected by nor treated for their disease. Older men could be spared a biopsy if a decision is made not to treat the cancer or if cancer progression would not substantially alter life expectancy.

TRUS-estimated volume is not always available to a physician. In the absence of TRUS-estimated volume prior to free PSA testing, volume-specific cutoffs would provide no clinical benefit. Analysis in this study showed that use of a single 25% fPSA cutoff was recommended regardless of prostate size: men above the cutoff would have large glands and the greatest likelihood of benign disease and so would not be biopsied. Men with cancer tend to have smaller prostate volumes and lower %fPSA values.

The correlation between %fPSA and volume could provide information to the physician on gland size and total PSA production relative to each other. If total PSA is elevated yet free PSA is not, the probability is high that a patient with these findings has a small gland with cancer. If total PSA is elevated and a large proportion of the total consists of free PSA,

then the probability is high that a patient with these findings has a large gland without cancer.

A single cutoff of 25% is recommended for men aged 50 to 75 years with PSA results between 4 and 10 ng/mL and benign findings on DRE, regardless of prostate size. This cutoff results in a high sensitivity in younger men and detects the most cancers with those adverse pathologic features that suggest aggressive tumor behavior.

The %fPSA values were significantly lower in cancer subjects than in benign subjects for both Caucasians and African-Americans, suggesting that %fPSA aids in distinguishing cancer from benign prostatic conditions in men of either race. %fPSA cutoffs, sensitivities, and specificities were not significantly different between groups, also suggesting that %fPSA performed in a similar manner in both Caucasian and African-American men.

The data were also analyzed to estimate an individual patient's probability of having cancer based on the %fPSA value and the patient's age. The risk estimates may also be used in discussions between physicians and patients regarding available options. The risk of prostate cancer for various %fPSA ranges was based on the previously reported 25% positive biopsy rate observed in a cancer detection population with PSA values of 4.0 to 10.0 ng/mL and a nonsuspicious rectal examination. If cancer probabilities were based upon the 50% proportion of cancer subjects in the current study, it could inflate the risk estimates for detecting cancer. The proportion of cancer subjects was adjusted from 50% to 25% prior to calculating cancer probabilities in order to provide accurate risk estimates appropriate for the group of men in whom this test would be used. An established statistical method, the bootstrap resampling method, was used to randomly sample the study population 1,000 times. Median cancer probabilities (risk estimates) and odds ratios were then calculated. This repetitive sampling increases the reliability of the risk estimates. These risk estimate results are shown in Table 8. A significant relationship was found between %fPSA and the relative risk of cancer in individual men. Lower %fPSA values indicate higher risk. Older men were at higher risk than younger men. The probability of cancer ranged from 5% to 56% for this study population, depending upon %fPSA value and patient age. Therefore, %fPSA, together with patient age added

useful information regarding an individual patient's risk and may be used as an aid in patient treatment options when candidate patients have similar characteristics as clinical trial patients. Table 8 represents the calculated risk estimates which physicians and patients may use in discussions regarding various options. Physicians could recommend biopsy for a patient with 5% fPSA who is 50 years old based on a cancer risk estimate of 56%. Physicians could recommend watchful waiting for a patient with 22% fPSA who is 73 years old based on the cancer risk estimate of 20%).

**Table 8**  
**Risk Assessment: Probability of Prostate Cancer**  
**(for Patients with PSA between 4 and 10 ng/mL)**

Percent Free PSA (%fPSA)	Patient Age	
	50 to 64 Years	65 to 75 Years
0.00 to 10.00%	56%	55%
10.01 to 15.00%	24%	35%
15.01 to 20.00%	17%	23%
20.01 to 25.00%	10%	20%
≥ 25.01%	5%	9%

The %fPSA may be used in two ways:

- 1) a single cutoff approach (all men with ≤ 25% fPSA would be recommended for followup); or
- 2) individual patient risk could be assessed to aid in biopsy decisions. Family and patient history and other factors, such as age, could be used in combination with %fPSA results to determine the best individual patient decision.

Prior studies have shown that in a prostate cancer detection program, approximately 9% of men over 50 years old would be identified as having non-suspicious DRE results and Tandem (total) PSA between 4 and 10 ng/mL. Free PSA and %fPSA would then be determined on these patients, and results would be used as an aid in patient biopsy decisions.

Study results were obtained using Hybritech's Tandem free PSA and (total) PSA assays. Recent studies have shown that combination of free and total PSA assays from different manufacturers can produce large differences in %fPSA values, raising issues of public health and safety.<sup>13-16</sup> The %fPSA cutoffs and clinical performance differ when various combinations of free and total PSA assays from different manufacturers are used. Mean %fPSA values from identical serum samples may be two-fold higher using different assay combinations,<sup>14</sup> and 95% sensitivity cutoffs may vary from 22% to 34% using different assay combinations.<sup>16</sup> For this reason, the clinical recommendations presented here apply only to the Tandem assays; results from other manufacturers may vary significantly and would require manufacturer-specific validation of clinical recommendations made.

#### Risk/Benefit Analysis

This study found that %fPSA significantly enhanced the specificity of testing with total PSA for prostate cancer detection. If a cutoff of  $\leq 25\%$  fPSA were used, 95% of the cancers would be detected and 20% of the men with benign disease would be spared biopsy. Since 75% of men with total PSA between 4 and 10 ng/mL and benign rectal examination findings have negative biopsy results, those spared biopsy represented numerous men.

Sparing men who do not need biopsy from the discomfort, costs, and anxiety of the procedure, as well as possible serious complications (such as infection, rectal bleeding, urinary retention and hospitalization) are clinical benefits.

Complications of biopsy occur, and serious complications are occasionally possible. Aside from the discomfort and anxiety, complications include infection, fever, rectal bleeding, blood in the urine or semen, urinary retention, and hospitalization in some cases. Sparing men who do not need biopsy from possible complications is an important clinical benefit.

The cancers above the cutoff, which would be missed, were more prevalent in older men. The missed cancers were more likely to be less aggressive, indolent tumors which would most likely not affect the patient's life.

For patients with borderline total PSA values (near 4 ng/mL) who might be hesitant to undergo prostatic biopsy, low %fPSA values may suggest the need for increased follow-up. Likewise, patients who have undergone one biopsy with negative findings might be advised to undergo a second biopsy if the %fPSA value suggests higher risk. Approximately 20% of cancers were missed on the first biopsy. In addition to enhancing specificity, %fPSA may enhance sensitivity in high risk patient populations and cancers would be detected that otherwise might be missed.

In conclusion, %fPSA as measured by Hybritech's Tandem assays enhanced the specificity of Tandem (total) PSA testing for prostate cancer detection with minimal loss in sensitivity. The %fPSA provided unique information regarding individual patient risk and may be used with other clinical parameters as an aid in patient management.

### **VIII. Conclusions**

The foregoing studies have demonstrated the safety and effectiveness of percent free PSA (Tandem free PSA / Tandem total PSA x 100) as measured by Tandem Assays as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection, in men aged 50 to 75 years with a total PSA between 4 and 10 ng/mL and digital rectal examination (DRE) findings that are not suspicious for cancer.

All of the Tandem®-R and Tandem®-MP free PSA assay performance specifications, including analytical sensitivity, analytical specificity, reproducibility, linearity and stability, are within acceptable limits for devices of this type.

The clinical trial demonstrated that the use of %fPSA as measured by the Tandem assays can increase the specificity of Tandem (total) PSA for prostate cancer detection in men with PSA levels of 4.0 to 10.0 ng/mL and benign findings on DRE. The resulting clinical benefit was a 20% reduction in unnecessary biopsies in men undergoing evaluation for prostate cancer, with minimal loss in overall cancer detection.

Therefore, FDA has concluded that the Tandem®-R and Tandem®-MP free PSA assays are safe and effective for the stated indication.

### ***IX. Panel Recommendation***

The Immunology Devices Panel recommended at the Panel meeting on February 2, 1998, that the PMA for the Tandem®-R and Tandem®-MP free PSA assays be approved.

### ***X. CDRH Action on the Application***

CDRH issued an approval order for the applicant's PMA for Hybritech's Tandem® free PSA and total PSA assays on March 10, 1998.

The applicant's manufacturing and control facilities were inspected in October, 1997 and the facilities were found to be in compliance with the Device Good Manufacturing Practice Regulations (GMPs). The shelf-life of Hybritech's Tandem® free PSA and total PSA assays has been established at 10 weeks for product stored between 2°C and 8°C.

### ***XI. Approval Specifications***

Directions for Use: See labeling

Conditions of Approval: FDA approval of this PMA is subject to full compliance with the conditions described in the approval order.

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## **Tandem®-R free PSA ImmunoRadioMetric Assay**

**For the Quantitative Measurement of  
free Prostate-Specific Antigen (free PSA) in Serum**

### **WARNING**

**The Tandem free PSA Assay should be used only with the Tandem (total) PSA Assay to calculate the ratio of free PSA to total PSA (percent free PSA). Use of another manufacturer's total PSA assay may result in:**  
**(1) an inappropriate population of patients selected for free PSA testing;**  
**and**

**(2) significantly different percent free PSA values, cutoffs and cancer probabilities than presented in the Expected Values section of this insert.**

**Results contained in this insert apply only to percent free PSA as measured by the Tandem free PSA and (total) PSA Assays.**

**The concentration of free PSA and total PSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must specify the manufacturer of the free and total PSA assays used. Values obtained with different manufacturers' assays cannot be used interchangeably.**

**Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by or on the order of a physician.**

### **INTENDED USE**

The Tandem-R *free* PSA Immunoradiometric Assay is an *In Vitro* device for the quantitative measurement of free prostate specific antigen (free PSA) in human serum.

Hybritech's Tandem-R *free* PSA is intended to be used with Tandem (total) PSA to calculate the ratio of free PSA to total PSA expressed as a percentage (percent free PSA).

Percent free PSA as measured by Hybritech's Tandem assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection in men aged 50 years or older with total PSA between 4 and 10 ng/mL and digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer.

## SUMMARY AND EXPLANATION OF THE TEST

Prostate cancer is the most common type of cancer found in men in the United States, with an incidence of approximately one case for every ten men; it is also the second leading cause of cancer deaths among American men.<sup>1</sup>

Prostate-specific antigen was identified and purified by Wang and co-workers in 1979.<sup>2</sup> PSA, a serine protease, is produced by the epithelial cells of the prostate, and is produced by both benign and malignant cells. Abnormalities in the prostate gland architecture resulting from trauma or disease can lead to "leakage" of PSA into the bloodstream.

PSA exists primarily as three forms in serum.<sup>3</sup> One form of PSA is believed to be enveloped by the protease inhibitor, alpha-2 macroglobulin<sup>4</sup> and has been shown to lack immunoreactivity. A second form is complexed to another protease inhibitor, alpha-1 antichymotrypsin (ACT).<sup>4,5</sup> The third form of PSA is not complexed to a protease inhibitor, and is termed free PSA.<sup>4,5</sup> The latter two forms are immunologically detectable in commercially available PSA assays and are referred to as total PSA.

Previous reports have shown that measurement of PSA forms is useful in the differentiation of prostate cancer from benign prostatic conditions.<sup>6,7</sup> In patients with elevated PSA concentrations, men with prostate cancer tend to have lower percent free PSA (free PSA/total PSA) values than men with benign disease.<sup>8-11</sup> This difference in the distribution of percent free PSA values in men with and without cancer may be used to select cutoffs for biopsy decisions, maintaining 90% to 95% sensitivity, while sparing 20% to 30% of men with benign disease from biopsy.

Percent free PSA may also be used for risk assessment, to determine the probability of cancer for an individual patient. Lower percent free PSA values are associated with higher risk of cancer.<sup>8-11</sup>

## PRINCIPLE OF PROCEDURE

The Tandem-R *free* PSA assay is a solid phase, two-site immunoradiometric assay. Samples containing free PSA are reacted with a plastic bead (solid phase) coated with a monoclonal antibody directed toward a particular site on the PSA molecule and with a radiolabeled monoclonal antibody directed against a distinct antigenic site on the free PSA molecule. Following the formation of a solid phase/free PSA/labeled antibody sandwich, the bead is washed to remove unbound labeled antibody. The radioactivity bound to the solid phase is measured in a gamma counter. The amount of radioactivity is directly proportional to the concentration of free PSA present in the test sample. The calculation of free PSA concentration in the sample is based on concurrent testing of the free PSA Calibrators from 0 to 20 ng free PSA/mL.

## REAGENTS / MATERIALS PROVIDED

### Antibody Set

Component	Cat. # 3818 100 Tests
<b><u>Anti-free PSA Tracer Antibody</u></b>	2 x 5 mL
Mouse monoclonal IgG (anti-free PSA) labeled with $^{125}\text{I}$ in a horse/mouse protein matrix containing less than 10 $\mu\text{Ci}$ or 370 kBq per vial, a blue dye and 0.1% sodium azide as a preservative.	
<b><u>Anti-PSA Coated Beads</u></b>	1 x 100 bds
Mouse monoclonal IgG (anti-PSA) coated on plastic beads, in a buffer containing 0.1% sodium azide as a preservative.	
<b><u>Zero Diluent/Calibrator (A)</u></b>	1 x 4 mL
A bovine protein matrix containing no detectable concentration of human free PSA (0 ng free PSA/mL) and 0.1% sodium azide as a preservative.	
<b><u>Free PSA Calibrators (B-F)</u></b>	5 x 2 mL
A bovine protein matrix containing approximately 0.5, 2, 5, 10 and 20 ng human free PSA/mL, a blue dye and 0.1% sodium azide as a preservative. Refer to insert label for assigned values.	
<b><u>Low free PSA Control (1)</u></b>	1 x 2 mL
Contains approximately 1.0 ng human free PSA/mL in a bovine protein matrix containing 0.1% sodium azide as a preservative. Refer to insert label for assigned range.	
<b><u>High free PSA Control (2)</u></b>	1 x 2 mL
Contains approximately 15 ng human free PSA/mL in a bovine protein matrix containing 0.1% sodium azide as a preservative. Refer to insert label for assigned range.	
<b><u>Wash Concentrate</u></b>	1 x 18 mL
Detergent solution containing 0.3% sodium azide as a preservative.	

## PRECAUTIONS

1. **For *In Vitro* diagnostic use only.**
2. **Radioactive Material:** This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories or hospitals, and only for in vitro clinical or laboratory tests not involving the internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the local regulations concerning radioactive substances.
3. **Radioactive Material for U.S.A. Only:**  
Follow the guidelines for receipt, acquisition, possession, use and transfer of the general license of the U.S.A. Nuclear Regulatory Commission or a state with which the commission has entered into an agreement for the exercise of regulatory authority. Users of this kit should adhere to these precautions or similar local precautions which have been established with consistency to U.S.A. Nuclear Regulatory Commission requirements.
  - a. Radioactive materials should be stored in the original container in a specially designated area. This storage area should be as far from the work area as practical.
  - b. Unused radioactive material from this kit may be disposed of by placing in a sink and flushing with a large quantity of water, unless local rules suggest otherwise.
  - c. Persons under 18 years of age should not be permitted to handle radioactive material or enter radioactive areas.
4. The State of California has adopted a regulation which requires a warning be given to individuals who may be exposed to chemicals identified by the State to cause cancer or reproductive harm. This product contains the radionuclide <sup>125</sup>I, which has been identified by the State of California to cause cancer. Accordingly, Hybritech advises you of the following warning:  
**Warning:** This product contains a chemical known to the State of California to cause cancer.
5. **Do not pipette by mouth.**
6. **Material should be used only in designated work areas with absorbent covering on the laboratory bench surfaces.**
7. **Wipe up any spills with an absorbent material, and wash the involved surface with a suitable detergent. Dispose of contaminated material properly.**
8. **Do not eat, drink or smoke in designated work areas.**
9. **Wear disposable gloves, laboratory coats, and other appropriate protective devices when handling radioactive material. Wash hands thoroughly after handling specimens and kit reagents.**
10. The Calibrators (B-F) and Controls (1-2) of this kit contain material of human origin which has been tested using FDA-approved methods and has been found negative for antibody to human immunodeficiency virus (HIV-I and HIV-II), antibody to Hepatitis C virus and for Hepatitis B surface antigen (HBsAG). No known test method can offer total assurance that HIV-I, HIV-II, Hepatitis B virus, Hepatitis C virus or other known infectious agents are absent. **Handle these**

reagents as if they were potentially infectious. Information on handling human serum is provided in the U.S.A. CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories" (HHS publication No. (NIH) 93-8395).

11. **HAMA Interference:** Some individuals have antibodies to mouse protein (HAMA), which can cause interference in immunoassays that employ antibodies derived from mice. In particular, it has been reported that serum samples from patients who have undergone therapy or diagnostic procedures that include infusion of mouse monoclonal antibody may produce erroneous results in such assays. Therefore, Tandem-R *free* PSA results for such patients should be used only in conjunction with results from some other diagnostic procedure and with information available from the clinical evaluation of the patient.
12. **Reagents in this kit contain sodium azide**, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Harmful if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water.
13. **Do not mix materials from different kit lots.**
14. **Do not use kit components beyond the expiration date.**
15. **Avoid microbial contamination of the reagents when removing aliquots from the vials.**

#### STORAGE AND STABILITY

- Tandem-R *free* PSA reagents are to be stored between 2°C and 8°C, at which temperatures they are stable until the expiration date printed on the box label.
- Wash Solution is stable at 2°C to 30°C until the expiration date of the kit. If reagent solution is turbid or contains precipitates, contact Hybritech Technical Support (EUROPE: 32 4 361 6363; U.S.A.: 1-800-854-1957).
- All reagents must be brought to room temperature (18°C to 25°C) prior to use. After use, all reagents except the Wash Solution should be stored at 2°C to 8°C.
- Recovery of the kit control concentrations should fall within the stated ranges.

#### SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is necessary. Specimens for free PSA testing should be drawn prior to such prostatic manipulations as digital rectal examination (DRE), prostatic massage, transrectal ultrasound (TRUS), and prostatic biopsy. DRE may cause a transient increase in both free and total PSA.<sup>12</sup> A repeat total PSA measurement in the case of borderline elevation is recommended.<sup>13</sup>

Transrectal needle biopsy has also been shown to cause transient increases in free PSA and persisting total PSA elevations,<sup>12,13</sup> thus a six-week waiting period between needle biopsy and PSA sampling has been recommended.

- Serum is required for the Tandem-R *free* PSA Assay. Plasma samples should **not** be used.
- Only blood drawn by an acceptable medical technique into a collection tube with no anticoagulants should be used. Specimens should be collected in such a way as to avoid hemolysis.

- The specimen should be allowed to clot and the serum separated by centrifugation. **Specimens should be processed (centrifuged) and refrigerated within 3 hours of blood draw.**
- If the serum sample is to be assayed within 24 hours after collection, the specimen should be stored in a refrigerator at 2°C to 8°C. Specimens held for longer times (up to 5 months) should be frozen at -20°C or colder.<sup>14,15</sup> Specimens to be held for longer than 5 months should be frozen at -70°C.<sup>14,15</sup> Repeated freeze-thaw cycles have no effect on free PSA, total PSA, or percent free PSA.<sup>14</sup> However, prompt refreezing of the thawed samples is recommended.
- Turbid serum samples or samples containing particulate matter should be centrifuged prior to assay.

#### **MATERIALS REQUIRED BUT NOT PROVIDED**

Plastic test tubes, round bottom, 12 X 75 mm

Test tube rack

Gamma counter

Repeating precision pipettors: 100 µL ( ± 1% )

Repeating pipettor: 2 mL

Disposable tip precision pipettors: 200 µL ( ± 1% )

Aspiration device (e.g., HybriWash®) or decant rack

Horizontal rotator (170 ± 10 rpm)

Distilled water

Forceps

Container for storage of Wash Solution

Pipet tips

#### **MATERIALS AVAILABLE FROM HYBRITECH**

Hybritech Bead Handling System      Cat. #2058

HybriWash® Bead Washing System      Cat. #2038B

Hybritech Bead Gun™      Cat. # 2036

Decant Top      Cat. #2015

Horizontal Rotator      Cat. #2041

#### **PREPARATION OF REAGENTS**

- Bring all reagents to room temperature (18°C to 25°C) prior to use.
- Thoroughly mix the reagents before each use by gentle agitation or swirling.
- Use a clean pipette or pipette tip for each specimen, Calibrator or Control to avoid contamination.
- Wash Solution: To prepare Wash Solution, add Wash Concentrate to 500 mL of distilled water and mix.

## ASSAY PROCEDURE

All Calibrators and Controls should be tested at the same time and run in duplicate. The assay procedure is as follows:

1. Label test tubes appropriately.
2. Pipette 200  $\mu$ L of Zero Diluent/Calibrator/Controls and specimens into each tube as labeled.
3. Introduce one Bead into each tube after blotting the residual droplet which remains on the Bead after removal from the container. Do not permit the Beads to dry.
4. Shake the test tube rack for 15 seconds to ensure mixing.
5. Pipette 100 $\mu$ L of Tracer Antibody into each tube and mix well.
6. Shake the test tube rack for 15 seconds to ensure mixing.
7. Incubate for 4 hours at room temperature on a horizontal rotator set at  $170 \pm 10$  rpm.
8. Wash the beads and tubes twice by:
  - a. Pipetting 2 mL of Wash Solution into each tube.
  - b. Aspirating the liquid from each tube. Alternatively, the Wash Solution may be removed by using a decant rack and decanting the Wash Solution after each wash.
9. Count each tube in a gamma counter and record the counts per minute.
10. Calculate the results as described in "Instrumentation and Calculation of Results."

## Procedural Notes

1. Samples with Tandem (total) PSA values greater than 1000 ng/mL may yield inaccurate results. These samples must be diluted before testing in the Tandem-R *free* PSA assay.
2. If a specimen was found to contain greater than 20 ng free PSA/mL, the specimen should be diluted with the Zero Diluent/Calibrator (A) and should be re-assayed according to the **Assay Procedure**. The dilution factor must be incorporated into the calculation of results. Each diluted specimen should be mixed thoroughly prior to testing. The recommended dilutions for specimens containing greater than 20 ng free PSA/mL are 1:5 or 1:10. However, it is desirable to dilute the serum specimens that contain more than 20 ng free PSA/mL so that the diluted sample reads greater than 0.5 ng free PSA/mL.
3. **Note on Bead Washing:** Immunometric assays require efficient washing to remove the unbound radiolabeled antibody. Therefore, it is very important to wash each tube efficiently, removing the last droplets of the Wash Solution to achieve optimal results. A squeezable, plastic wash bottle, Cornwall-type syringe or repipettor may be used to wash the walls of the tube and the Bead. Dispense the Wash Solution into each tube with sufficient force to "float" the Bead at least 2.5 cm from the bottom of the test tube and up into the Wash Solution. The Wash Solution should be removed thoroughly using either an aspirating device or a decant rack. The inverted decant rack should be blotted on absorbent paper after the final wash.

4. For convenience, repeating or multi-channel pipettors may be used for pipetting Tracer Antibody and Wash Solution. Pipettes with disposable tips are recommended for pipetting the Calibrators, Controls and specimens. The pipette tips should be changed after each sample is pipetted to avoid potential sample carryover and contamination of the reagents or specimens.

## **INSTRUMENTATION AND CALCULATION OF RESULTS**

Results may be calculated by using computer-assisted methods or manually on linear graph paper.

### **Computer-Assisted Method**

Computer-assisted data reduction may be used to calculate results for the Tandem-R *free* PSA Assay. Several curve-fitting methods can provide satisfactory results:

1. Point-to-Point: Software that connects a straight line between means of Calibrator replicates, including the Zero Diluent/Calibrator, provides good results with any of the procedures and calibration methods described.
2. Curve-fitting routines: These programs include polygonal interpolation, spline interpolation and modified spline interpolation, which may be used with linear, as well as nonlinear, calibration curves.

For additional information on computer-assisted data reduction, consult your local Sales Representative.

### **Manual Method**

The Tandem-R *free* PSA curve may be constructed manually on linear graph paper by plotting the counts per minute (CPM) for each Calibrator replicate on the y-axis versus the concentration of free PSA on the x-axis. The best fit curve should be drawn through the calibration points. Do not force the curve to a straight line.

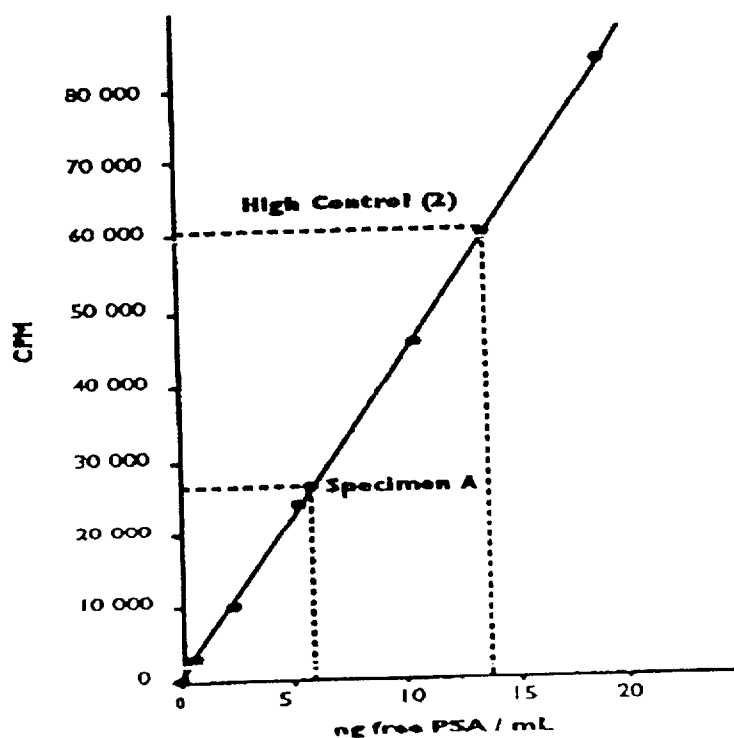
To determine the concentration of free PSA in the Controls and patient specimens, extend a horizontal line from the CPM value for the test sample to the calibration curve. At the point of intersection of the horizontal line and the curve, drop a vertical line to the x-axis and read the concentration of free PSA in ng/mL.

If the sample was diluted prior to assaying, the observed free PSA concentration must be multiplied by the dilution factor.

### Example Data

Well #	Description	CPM	Mean CPM	ng/mL
1	Zero Calibrator (A)	423	443	-----
2	" "	463		
3	0.5 ng/mL Calibrator (B)	2888	2905	-----
4	" "	2921		
5	2 ng/mL Calibrator (C)	10014	10050	-----
6	" "	10086		
7	5 ng/mL Calibrator (D)	24611	25000	-----
8	" "	25388		
9	10 ng/mL Calibrator (E)	46383	45889	-----
10	" "	45394		
11	20 ng/mL Calibrator (F)	83200	83545	-----
12	" "	83890		
13	Low Control (1)	4633	4677	0.88
14	" "	4722		
15	High Control (2)	60942	60469	14.34
16	" "	59996		
17	Specimen A	27961	27670	5.72
18	" "	27379		

### Example Curve



### Percent free PSA

Free PSA values alone have not been shown to be effective in patient management and should not be used. Both total PSA and free PSA concentrations should be determined on the same serum specimen and used to calculate the percentage of free PSA. Percent free PSA values are then used for patient management.

$$\frac{\text{Tandem free PSA (ng/mL)}}{\text{Tandem (total) PSA (ng/mL)}} \times 100\% = \text{Percent free PSA}$$

### EXPECTED VALUES

A multi-center, prospective clinical trial was conducted to test the effectiveness of percent free PSA as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection.

All subjects were between 50 and 75 years of age, with serum PSA values between 4 and 10 ng/mL and digital rectal examination (DRE) findings that were not suspicious for cancer. These men represent the "diagnostic gray zone", in which total PSA has identified the men as high risk (25% cancer rate compared to a 4% cancer rate for the general population of men over 50 years of age), but where specificity could be improved. All men had undergone ultrasound-guided six-sector needle biopsies of the prostate, and thus had a histologically-confirmed diagnosis prior to determination of free PSA concentrations. The study was blinded; pathologists did not have access to percent free PSA values, and laboratorians did not have access to diagnoses. Exclusion criteria included acute prostatitis, urinary tract infection, prior transurethral resection of the prostate (TURP), or recent prostatic manipulation or medications that might alter serum PSA concentrations.

A total of 773 men participated in the study. Median age for both cancer and benign disease subjects was 64 years. The study population was 86% Caucasian, 9% African-American, 3% Hispanic, and 2% Asian.

Table 1 shows the expected values for free PSA (ng/mL), total PSA (ng/mL), and percent free PSA [(free PSA / total PSA) x 100%] for this population of men.

**Table 1**  
**Tandem free PSA (ng/mL), Total PSA (ng/mL) and Percent free PSA (%):**  
**Expected Values, by Diagnosis**

		<b>Benign N = 394</b>	<b>Cancer N = 379</b>	<b>Total N = 773</b>
<b>Free PSA</b>	<b>Median</b>	1.0	0.7	0.9
	<b>Mean <math>\pm</math> SD</b>	1.1 $\pm$ 0.6	0.8 $\pm$ 0.5	1.0 $\pm$ 0.6
	<b>Range</b>	0.2 - 4.9	0.2 - 3.6	0.2 - 4.9
<b>Total PSA</b>	<b>Median</b>	5.6	5.9	5.8
	<b>Mean <math>\pm</math> SD</b>	6.0 $\pm$ 1.6	6.2 $\pm$ 1.7	6.1 $\pm$ 1.6
	<b>Range</b>	4.0 - 10.0	4.0 - 10.0	4.0 - 10.0
<b>% free PSA</b>	<b>Median</b>	17.9	12.2	15.3
	<b>Mean <math>\pm</math> SD</b>	19.0 $\pm$ 7.8	13.4 $\pm$ 6.8	16.3 $\pm$ 7.9
	<b>Range</b>	4.3 - 52.2	2.3 - 42.1	2.3 - 52.2

In a prostate cancer detection program, DRE and PSA testing would identify men with non-suspicious DRE results and PSA between 4 and 10 ng/mL. Free PSA and percent free PSA would then be determined on these patients, and results would be used as an aid in patient management.

The multi-center clinical trial results demonstrated that percent free PSA may be used in two ways:

- (1) individual patient risk assessment to aid in management decisions; or
- (2) a single cutoff (men with values less than or equal to a certain cutoff would be candidates for additional follow-up procedures such as biopsy).

### Individual Patient Risk Assessment

Percent free PSA may be used to determine the relative risk of prostate cancer in individual men. Family and patient history can be used in combination with percent free PSA results to determine the best individualized patient management decisions.

Table 2 shows the probability of detecting prostate cancer with needle biopsy, based on total PSA and percent free PSA results. PSA results in this table were obtained from a prior multi-center study evaluating the efficacy of total PSA for prostate cancer detection,<sup>16,17</sup> and percent free PSA results were obtained from the current study.

It can be seen that rising PSA levels increase the risk of detectable cancer. Percent free PSA can further stratify risk for men with PSA values between 4 and 10 ng/mL and non-suspicious digital rectal examination results. Lower percent free PSA values indicate higher risk. The risk of cancer ranged from 8% to 56% for this population. For purposes of comparison, the risk of prostate cancer is 4% for the general population of men over 50 years of age.<sup>16</sup>

**Table 2.**  
Probability of Prostate Cancer, Based on PSA and Percent Free PSA Results  
(for Men with Non-Suspicious DRE Results, Regardless of Patient Age)

PSA	Probability of Cancer	Percent free PSA	Probability of Cancer
0-2 ng/mL	1%	0-10%	56%
2-4 ng/mL	15%	10-15%	28%
4-10 ng/mL	25%	15-20%	20%
>10 ng/mL	>50%	20-25%	16%
		>25%	8%

Percent free PSA values should not be interpreted as definitive evidence for the presence or absence of prostate cancer. Prostatic biopsy is required for diagnosis of cancer.

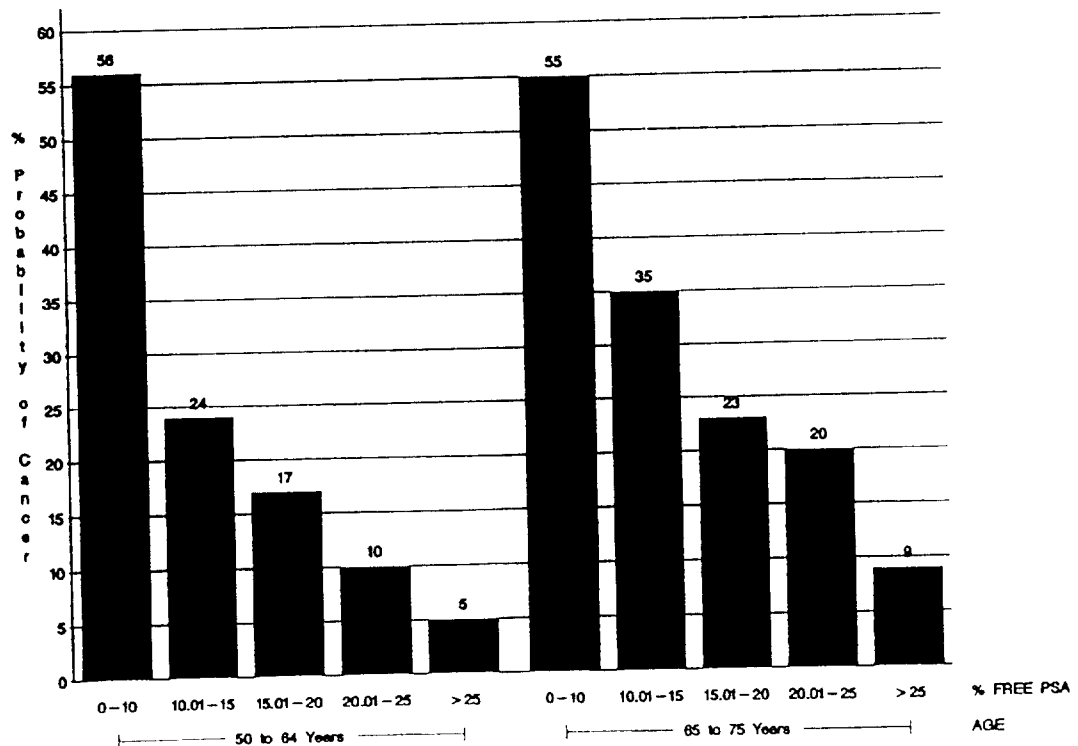
The clinical trial results also demonstrated that older men were at higher risk than younger men. The probability of cancer by percent free PSA value and age is shown in Table 3 and Figure 1.

**Table 3**  
**Probability of Prostate Cancer**  
**(for Men with Non-Suspicious DRE Results**  
**and PSA between 4 and 10 ng/mL, by Patient Age)**

Percent free PSA (%FPSA)	Patient Age	
	50 to 64 years	65 to 75 years
0.00 to 10.00%	56%	55%
10.01 to 15.00%	24%	35%
15.01 to 20.00%	17%	23%
20.01 to 25.00%	10%	20%
≥ 25.01%	5%	9%

**Figure 1**

Probability of Cancer  
by Percent Free PSA and Patient Age  
for Patients with PSA between 4 and 10 ng/mL



## Single Cutoff

Rather than using risk assessment, a cutoff approach to patient management can also be used. Table 4 shows sensitivity (percentage of cancers detected) and specificity (percentage of biopsies avoided in men without cancer) for various percent free PSA cutoffs. A cutoff of  $\leq 25\%$  free PSA was selected based on data from the clinical trial. When men with values of 25% free PSA or less were biopsied, 95% of cancers were detected. The majority of men with PSA values between 4 and 10 ng/mL have benign disease. In this clinical trial, 20% of biopsied men with benign disease and a percent free PSA value greater than the 25% free PSA cut-off could have been spared from biopsy.

The cutoff of  $\leq 25\%$  free PSA is based on results from this clinical trial. Additional follow-up may be recommended for men with percent free PSA values above this cutoff if the physician believes it is necessary based upon other factors in the patient's medical or family history.

**Table 4.**  
**Sensitivity and Specificity for Various Percent free PSA (%FPSA) Cutoffs**  
**Recommended Cutoff:  $\leq 25\%$  FPSA**  
**(Biopsy men with values less than or equal to this cutoff).**

%FPSA Cutoffs	Sensitivity (# of cancers detected/ # of total cancers)			Specificity (# of non-cancers detected/ # of total non-cancers)		
	%	(n/N)	95% CI *	%	(n/N)	95% CI *
$\leq 25\%$	95%	(358/379)	92 - 97%	20%	(80/394)	16 - 24%
$\leq 32\%$	98%	(373/379)	96 - 99%	6%	(25/394)	4 - 9%
$\leq 55\%$	100%	(379/379)		0%	(0/394)	

\* 95% CI = 95% Confidence Intervals

Table 5 shows that the cancers occurring in men with a percent free PSA value above the 25% cutoff (i.e. those cancers which would be missed if men above the cutoff were not biopsied) are found primarily in older men with larger glands. Older men (those with less than a 10 year life expectancy) are often not affected by nor treated for prostate cancer. Thus, use of percent free PSA would result in a recommendation for biopsy in younger men, those most likely to gain from early detection.

The volume finding is clinically advantageous. Men with percent free PSA values near and above the cutoff tend to have large glands (benign prostatic hyperplasia), whereas men with cancer have lower percent free PSA values which tend to cluster progressively further away from the cutoff. Thus, when the recommendation is made not to biopsy men above the cutoff, this is the group with the lowest risk of cancer and the highest probability of benign disease (see Table 2 and discussion in previous section, "Individual Patient Risk Assessment").

**Table 5.**  
**Characteristics of Cancer Subjects Above and Below Cutoff:**  
**Patient Age and Prostate Volume**

Percent free PSA Cutoff	Median Patient Age	Median Prostate Volume
> 25% free PSA	68 years	48 cc
≤ 25% free PSA	63 years	34 cc

#### **ACCEPTABILITY OF RESULTS**

- The mean CPM value of the Zero Diluent/Calibrator should be less than 1000.
- Recovery of the control concentrations should fall within the stated ranges.

#### **QUALITY CONTROL**

Good laboratory practice includes the use of control specimens within an assay run to ensure that all reagents and protocols are performing properly. The Tandem-R free PSA kit contains Controls which can be used to verify assay performance. The coefficient of variation for the Calibrators should be less than 10%.

## LIMITATIONS

Serum PSA concentrations (free, total, or percent free PSA) should not be interpreted as absolute evidence for the presence or absence of prostate cancer. Elevated total PSA concentrations or decreased percent free PSA may be observed in the serum of patients with non-malignant disorders, as well as those with prostate cancer. Furthermore, low total PSA concentrations or elevated percent free PSA are not necessarily indicative of the absence of cancer. Serum free and total PSA values should be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as digital rectal examination (DRE). Some cases of early prostate cancer will not be detected by PSA testing; the same is true for DRE. Biopsy of the prostate is the standard method used to confirm the presence or absence of prostate cancer.

The 5 alpha-reductase inhibitor drugs may affect PSA levels in some patients. Other drugs used to treat BPH may also affect PSA levels. Care should be taken in interpreting results from patients taking these drugs.

## PERFORMANCE CHARACTERISTICS

### Precision

Overall precision of the Tandem-R *free* PSA assay was determined by duplicate analyses of 5 free PSA controls across 20 runs. The data presented were calculated based on NCCLS EP5-T2 guidelines.

Sample	Mean (ng/mL)	Within-Run (%C.V.)	Between-Run (%C.V.)	Total (%C.V.)
1	0.42	4.02	5.44	6.76
2	1.03	3.29	3.39	4.73
3	2.00	1.85	3.64	4.09
4	4.08	1.08	2.87	3.06
5	15.47	1.89	1.13	2.20

### Inter-Laboratory Precision

Inter-laboratory precision was evaluated in 3 laboratories by assaying in triplicate a panel of serum samples spanning the assay range. A table representing the results obtained with 5 of the samples is provided below.

Sample	Mean (ng/mL)	Standard Deviation	% CV
1	0.32	0.01	3.13
2	1.05	0.01	0.55
3	2.05	0.03	1.29
4	6.24	0.13	2.13
5	15.63	0.09	0.57

### Dilution

Twelve serum samples containing elevated free PSA concentrations were diluted with Zero Diluent/Calibrator and assayed in quadruplicate at multiple dilutions. Free PSA concentrations observed versus concentrations expected were analyzed by linear regression. The correlation coefficients (*r*) varied between 0.9972 and 0.9999.

### Interfering Substances

- **Hemoglobin** and **bilirubin**, which were tested at concentrations up to 500 mg/dL and 20 mg/dL, respectively, do not interfere with the Tandem-R *free* PSA Assay.
- **Triglycerides**, which were tested at concentrations up to 2500 mg/dL, do not interfere with the Tandem-R *free* PSA Assay.
- When **total protein** was tested at concentrations between 4 and 13 g/dL, no significant effect on the results was observed.
- Cross-reactivity with **PSA-ACT** was determined to be less than 1%.

### Commonly Used Drugs

Various concentrations of drugs were added to serum samples containing free PSA and assayed in replicates of 4. The drugs and the highest concentrations tested are:

acetaminophen	0.2 mg/mL
aspirin	0.5 mg/mL
ibuprofen	0.4 mg/mL
naproxin sodium	1.0 mg/mL
hydrocodone	240 ng/mL
cimetidine	0.1 mg/mL
capotril	4.0 µg/mL
lovastatin	270 ng/mL
nifedipine	270 ng/mL
metoprolol	2.7 µg/mL
finasteride	370 ng/mL
terazosin hydrochloride	1.45 mg/mL
ciprofloxacin	46 µg/mL

sulfamethoxazole	116.5 µg/mL (in combination with)
trimethoprim	9.7 µg/mL
doxycycline	2.6 µg/mL
clomipramine HCl	2.7 µg/mL
fluoxetine	0.55 µg/mL
furosemide	20 µg/mL
methotrexate	13.2 µg/mL
prednisone	1.65 µg/mL
tavist-1	2.7 µg/mL

At the concentrations listed above, these drugs did not interfere with the recovery of free PSA from the serum samples.

### **Minimum Detectable Concentration**

The minimum detectable concentration of Tandem-R *free* PSA is less than 0.05 ng/mL. The minimum detectable concentration is defined as that concentration of free PSA that corresponds to the CPM that is two standard deviations greater than the mean CPM of 21 replicate determinations of the Zero Diluent/Calibrator (A) using Tracer at expiration of the kit.

### **Trouble-Shooting Guide**

Occasionally one may encounter poor reproducibility or high counts for the Zero Diluent/Calibrator; possible causes and solutions are provided below.

- |           |  |
|-----------|--|
| Cause:    | Insufficient washing of the Beads and tubes.   |
| Solution: | Ensure proper washing technique. (See Procedural Notes #3).  |
| Cause:    | Cross-contamination of the Calibrators, Controls and/or patient specimens  |
| Solution: | Ensure that proper pipetting techniques with minimal carryover are employed.   |
| Cause:    | Contamination of the gamma counter tubes or wells.   |
| Solution: | Count the background in the gamma counter tubes or wells and decontaminate according to manufacturer's recommendation. |
| Cause:    | Insufficient mixing of reaction tubes prior to initiation of incubation.   |
| Solution: | Ensure that proper technique is employed.  |

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Purchase of this kit licenses its use under U.S.A. Patent Nos. 4,376,110, 4,486,530 and 5,501,983.

Tandem is a trademark of Hybritech Incorporated.

Note: Not all catalog items are available in each country. Please contact your local representative for information concerning availability in your area.

Manufactured by: Hybritech Incorporated San Diego, CA 92121 U.S.A.

For additional information, please contact Hybritech Technical Support:

U.S.A. : 1-800-854-1957  
EUROPE : 32 4 361 6363

HYBRITECH INCORPORATED, San Diego, CA 92121 U.S.A.  
HYBRITECH EUROPE S.A. B - 4031 Liege, Belgium

Hybritech Incorporated, February 1998

**Tandem®-MP free PSA  
ImmunoEnzyMetric Assay**

For the Quantitative Measurement of  
**free Prostate-Specific Antigen (free PSA) in Serum**

**WARNING**

The Tandem free PSA Assay should be used only with the Tandem (total) PSA Assay to calculate the ratio of free PSA to total PSA (percent free PSA). Use of another manufacturer's total PSA assay may result in:  
(1) an inappropriate population of patients selected for free PSA testing; and  
(2) significantly different percent free PSA values, cutoffs and cancer probabilities than presented in the Expected Values section of this insert.

Results contained in this insert apply only to percent free PSA as measured by the Tandem free PSA and (total) PSA Assays.

The concentration of free PSA and total PSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must specify the manufacturer of the free and total PSA assays used. Values obtained with different manufacturers' assays cannot be used interchangeably.

Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by or on the order of a physician.

**INTENDED USE**

The Tandem-MP free PSA Immunoenzymetric Assay is an In Vitro device for the quantitative measurement of free prostate specific antigen (free PSA) in human serum.

Hybritech's Tandem-MP free PSA is intended to be used with Tandem (total) PSA to calculate the ratio of free PSA to total PSA expressed as a percentage (percent free PSA).

Percent free PSA as measured by Hybritech's Tandem assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection in men aged 50 years and older with total PSA between 4 and 10 ng/mL and digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer.

## SUMMARY AND EXPLANATION OF THE TEST

Prostate cancer is the most common type of cancer found in men in the United States, with an incidence of approximately one case for every ten men; it is also the second leading cause of cancer deaths among American men.<sup>1</sup>

Prostate-specific antigen was identified and purified by Wang and co-workers in 1979.<sup>2</sup> PSA, a serine protease, is produced by the epithelial cells of the prostate, and is produced by both benign and malignant cells. Abnormalities in the prostate gland architecture resulting from trauma or disease can lead to "leakage" of PSA into the bloodstream.

PSA exists primarily as three forms in serum.<sup>3</sup> One form of PSA is believed to be enveloped by the protease inhibitor, alpha-2 macroglobulin<sup>4</sup> and has been shown to lack immunoreactivity. A second form is complexed to another protease inhibitor, alpha-1 antichymotrypsin (ACT).<sup>4,5</sup> The third form of PSA is not complexed to a protease inhibitor, and is termed free PSA.<sup>4,5</sup> The latter two forms are immunologically detectable in commercially available PSA assays and are referred to as total PSA.

Previous reports have shown that measurement of PSA forms is useful in the differentiation of prostate cancer from benign prostatic conditions.<sup>6,7</sup> In patients with elevated PSA concentrations, men with prostate cancer tend to have lower percent free PSA (free PSA/total PSA) values than men with benign disease.<sup>8-11</sup> This difference in the distribution of percent free PSA values in men with and without cancer may be used to select cutoffs for biopsy decisions, maintaining 90% to 95% sensitivity, while sparing 20% to 30% of men with benign disease from biopsy.

Percent free PSA may also be used for risk assessment, to determine the probability of cancer for an individual patient. Lower percent free PSA values are associated with higher risk of cancer.<sup>8-11</sup>

## PRINCIPLE OF PROCEDURE

The Tandem-MP *free* PSA assay is a solid phase, two-site immunoenzymetric assay. Samples containing free PSA are reacted with a solution containing two PSA-specific monoclonal antibodies. One is enzyme-labeled anti-free PSA antibody; the other is biotin-labeled anti-PSA antibody. The reaction takes place in a plastic microplate (solid phase) consisting of a frame and several strips of wells coated with streptavidin (which binds with biotin). Following the formation of a solid phase/capture antibody/free PSA/labeled antibody sandwich, the microplate is washed to remove unbound labeled antibody and is then incubated with an enzyme substrate. The amount of substrate turnover is determined colorimetrically by measuring the absorbance of the quenched reaction at 450 nm in a microplate reader. The absorbance is proportional to the concentration of free PSA present in the test sample. The calculation of free PSA concentration in the sample is based on concurrent testing of the free PSA Calibrators from 0 to 20 ng free PSA/mL.

## REAGENTS / MATERIALS PROVIDED

Tandem-MP *free* PSA reagents are provided in two separate reagent sets. The Antibody Set provides the microplate strips and frames, immunochemicals, Calibrators, and Controls required to form the solid phase/capture antibody/free PSA/labeled antibody sandwich and calibrate the assay up to 20 ng free PSA/mL. The Liquid Substrate/Quench Set provides the reagents for the development of a color change measurable in a microplate reader at 450 nm.

## Antibody Set

Component	Cat. #4607 96 Tests
<b><u>Assay Conjugate</u></b> Anti-free PSA mouse monoclonal IgG conjugated to bovine alkaline phosphatase and anti-PSA mouse monoclonal IgG conjugated to biotin in a bovine/mouse protein matrix containing 0.1% sodium azide as a preservative.	1 x 9 mL
<b><u>Microplate</u></b> Streptavidin-coated plastic well strips in a plastic tray. Dessicant: silica gel	1 x 96 wells
<b><u>Zero Diluent/Calibrator (A)</u></b> A bovine protein matrix containing no detectable concentration of human free PSA (0 ng free PSA/mL) and 0.1% sodium azide as a preservative.	1 x 2 mL
<b><u>Free PSA Calibrators (B-F)</u></b> A bovine protein matrix containing approximately 0.5, 2, 5, 10 and 20 ng human free PSA/mL, a blue dye and 0.1% sodium azide as a preservative. Refer to insert label for assigned values.	5 x 1 mL
<b><u>Low free PSA Control (1)</u></b> Contains approximately 1.0 ng human free PSA/mL in a bovine protein matrix containing 0.1% sodium azide as a preservative. Refer to insert label for assigned range.	1 x 1 mL
<b><u>High free PSA Control (2)</u></b> Contains approximately 15 ng human free PSA/mL in a bovine protein matrix containing 0.1% sodium azide as a preservative. Refer to insert label for assigned range.	1 x 1 mL
<b><u>Wash Concentrate</u></b> Detergent solution containing 15% sodium chloride and 0.3% sodium azide as a preservative.	1 x 20 mL

**LIQUID SUBSTRATE / QUENCH SET (Provided Separately)****Component****CAT. #4020****Substrate Concentrate****1 x 3 mL**

p-Nitrophenyl phosphate in a stabilizing buffer containing preservatives.

**Substrate Diluent****1 x 30 mL**

Buffer containing preservatives.

**Quench Reagent****1 x 250 mL**

EDTA in a buffer containing a preservative.

**PRECAUTIONS**

1. **For In Vitro diagnostic use only.**
2. **Do not pipette by mouth.**
3. **Do not eat, drink or smoke in designated work areas.**
4. **Wash hands thoroughly after handling specimens and kit reagents.**
5. The Calibrators (B-F) and Controls (1-2) of this kit contain material of human origin which has been tested using FDA-approved methods and has been found negative for antibody to human immunodeficiency virus (HIV-I and HIV-II), antibody to Hepatitis C virus and for Hepatitis B surface antigen (HBsAG). No known test method can offer total assurance that HIV-I, HIV-II, Hepatitis B virus, Hepatitis C virus or other known infectious agents are absent. **Handle these reagents as if they were potentially infectious.** Information on handling human serum is provided in the U.S.A. CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories" (HHS publication No. (NIH) 93-8395).
6. **HAMA Interference:** Some individuals have antibodies to mouse protein (HAMA), which can cause interference in immunoassays that employ antibodies derived from mice. In particular, it has been reported that serum samples from patients who have undergone therapy or diagnostic procedures that include infusion of mouse monoclonal antibody may produce erroneous results in such assays. Therefore, Tandem-MP *free* PSA results for such patients should be used only in conjunction with results from some other diagnostic procedure and with information available from the clinical evaluation of the patient.
7. **Reagents in this kit contain sodium azide**, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Harmful if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water.

8. Do not mix materials from different kit lots.
9. Do not use kit components beyond the expiration date.
10. Avoid microbial contamination of the reagents when removing aliquots from the vials.

#### STORAGE AND STABILITY

- Tandem-MP *free* PSA reagents are to be stored between 2°C and 8°C, at which temperatures they are stable until the expiration date printed on the box label.
- The Substrate Concentrate as well as the combined Substrate Reagent are yellow. Combined Substrate Reagent is stable for 7 days when stored in the dark between 2°C and 8°C. The combined Substrate Reagent must not be used if the absorbance at 450 nm is greater than 0.11 units.
- Wash Solution is stable at 2°C to 30°C until the expiration date of the kit.
- If reagent solution is turbid or contains precipitates, contact Hybritech Technical Support (EUROPE: 32 4 361 6363; U.S.A.: 1-800-854-1957).
- All reagents must be brought to room temperature (18°C to 25°C) prior to use. After use, all reagents except the Wash Solution should be stored at 2°C to 8°C.
- Recovery of the kit control concentrations should fall within the stated ranges.
- Unused well strips should be returned to the plastic storage tray with the desiccant pouch provided and stored at 2°C to 8°C.

#### SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is necessary. Specimens for free PSA testing should be drawn prior to such prostatic manipulations as digital rectal examination (DRE), prostatic massage, transrectal ultrasound (TRUS), and prostatic biopsy. DRE may cause a transient increase in both free and total PSA.<sup>12</sup> A repeat total PSA measurement in the case of borderline elevation is recommended.<sup>13</sup>

Transrectal needle biopsy has also been shown to cause transient increases in free PSA and persisting total PSA elevations,<sup>12,13</sup> thus a six-week waiting period between needle biopsy and PSA sampling has been recommended.

- Serum is required for the Tandem-MP *free* PSA Assay. Plasma samples should **not** be used.
- Only blood drawn by an acceptable medical technique into a collection tube with no anticoagulants should be used. Specimens should be collected in such a way as to avoid hemolysis.
- The specimen should be allowed to clot and the serum separated by centrifugation. **Specimens should be processed (centrifuged) and refrigerated within 3 hours of blood draw.**
- If the serum sample is to be assayed within 24 hours after collection, the specimen should be stored in a refrigerator at 2°C to 8°C. Specimens held for longer times (up to 5 months) should be frozen at -20°C or colder.<sup>14,15</sup> Specimens to be held for longer than 5 months should be frozen at -70°C.<sup>14,15</sup> Repeated freeze-thaw cycles have no effect on free PSA, total PSA, or percent free PSA.<sup>14</sup> However, prompt refreezing of the thawed samples is recommended.
- Turbid serum samples or samples containing particulate matter should be centrifuged prior to assay.

## **MATERIALS REQUIRED BUT NOT PROVIDED**

Liquid Substrate/Quench Set CAT.#4020/4021

Liquid Substrate CAT.#4022

Disposable tip precision pipettors: 50  $\mu$ L (  $\pm$  1% )

Repeating pipettors: 50, 100 and 300  $\mu$ L

Disposable pipette tips for 50, 100 and 300  $\mu$ L

Test tubes for sample dilutions

Microplate washer

Aspiration device

Distilled water

Timer

Container for storage of Wash Solution

Container for preparing Substrate Reagent

Microplate reader capable of reading at 450 nm, linear to 3.0 abs. units

Horizontal microplate rotator (800  $\pm$  100 rpm) - orbit 3 mm

Contact Hybritech Technical Support for assistance with other settings.

## **PREPARATION OF REAGENTS**

- Bring all reagents to room temperature (18°C to 25°C) prior to use.
- Thoroughly mix the reagents before each use by gentle agitation or swirling.
- Use a clean pipette or pipette tip for each specimen, Calibrator or Control to avoid contamination.
- Wash Solution: To prepare Wash Solution, add Wash Concentrate to 600 mL of distilled water and mix.
- Substrate Reagent: Dilute 1 vial (3.0 mL) of Substrate Concentrate by pouring entire contents into 1 vial (30 mL) of Substrate Diluent. Alternatively, to prepare smaller volumes, dilute 1 volume of Substrate Concentrate into 10 volumes of Substrate Diluent.

## **ASSAY PROCEDURE**

The procedure for the Tandem-MP *free* PSA assay is performed at room temperature. Bring all serum specimens and kit components to room temperature (18°C to 25°C) and mix well before use.

All Calibrators and Controls should be tested at the same time and run in duplicate. Because the termination of each incubation stops a reaction that is in progress (i.e., antibody binding or substrate turnover), reliable calibration of the assay depends on ensuring that the incubation times are essentially the same for all of the wells.

The assay procedure is as follows:

1. Label test well strips and holders appropriately and load strips into holder frame. Run each of 6 calibrators, low and high controls, and patient specimens for each assay.
2. Pipette 50  $\mu$ L of Zero Diluent/Calibrator/Controls and patient specimens into each well as labeled.
3. Pipette 50  $\mu$ L of Assay Conjugate into each well.
4. Incubate for 1 hour at room temperature (18°C to 25°C) using a horizontal rotator set at 800 rpm  $\pm$  100 rpm.
5. Wash the microplate wells 3 times by:
  - a. Aspirating the samples from each of the wells.
  - b. Pipetting 300  $\mu$ L of Wash Solution into each well.
  - c. Aspirating the liquid from each well.
  - d. Repeating steps b and c twice.
6. Dispense 100  $\mu$ L of Substrate Reagent into each well.
7. Incubate for 30 minutes at room temperature (18°C-25°C) using a horizontal rotator set at 800 rpm  $\pm$  100 rpm.
8. Dispense 100  $\mu$ L of Quench Reagent into each well.
9. Read the absorbance of each well at 450 nm in a microplate reader. Read the plate within 1 hour of quenching the reaction.
10. Calculate the results as described in "Instrumentation and Calculation of Results."

#### Procedural Notes

1. Samples with Tandem (total) PSA values greater than 200 ng/mL may yield inaccurate results. These samples must be diluted before testing in the Tandem-MP *free* PSA assay.
2. If a specimen was found to contain greater than 20 ng free PSA/mL, the specimen should be diluted with the Zero Diluent/Calibrator (A) and should be reassayed according to the **Assay Procedure**. The dilution factor must be incorporated into the calculation of results. Each diluted specimen should be mixed thoroughly prior to testing. The recommended dilutions for specimens containing greater than 20 ng free PSA/mL are 1:5 or 1:10. However, it is desirable to dilute the serum specimens that contain more than 20 ng free PSA/mL so that the diluted sample reads greater than 0.5 ng free PSA/mL.
3. **Note on Plate Washing:** Immunometric assays require efficient washing to remove the unbound labeled antibody. Therefore, it is very important to wash each well efficiently, removing the last droplets of the Wash Solution to achieve optimal results.
4. Because absorbance is a function of temperature and duration of the Substrate Reagent incubation, it is very important that this incubation be the same for all wells/plate. This can be accomplished by ensuring that the elapsed time for pipetting reagents from beginning to end (without interruption) is the same  $\pm$  2 minutes for both the Substrate Reagent addition step and the Quench Reagent addition step.

5. For convenience, repeating or multichannel pipettors may be used for dispensing Assay Conjugate, Wash Solution, Substrate and Quench Reagent. Pipettors with disposable tips are recommended for pipetting Calibrators, Controls and specimens. The pipette tips should be changed after each sample is pipetted to avoid potential sample carryover and contamination of the reagents or specimens.
6. Note on Plate Rotation: Accurate rotation speed is important for accurate results. The plates must rotate at a speed such that the fluid in each well clearly forms a vortex but does not rise above the upper edge of the wells.
7. Note on Plate Reading: Measuring and subtracting absorbances at a reference wavelength (600-650 nm) from each well may enhance assay precision.

## **INSTRUMENTATION AND CALCULATION OF RESULTS**

Results may be calculated by using computer-assisted methods or manually on linear graph paper.

### **Computer-Assisted Method**

Computer-assisted data reduction may be used to calculate results for the Tandem-MP *free* PSA Assay. A point-to-point curve fitting routine, software that connects a straight line between means of Calibrator replicates, including the Zero Diluent/Calibrator, provides acceptable results.

For additional information on computer-assisted data reduction, consult your local Sales Representative.

### **Manual Method**

The Tandem-MP *free* PSA calibration curve generated at 450 nm may be constructed manually on linear graph paper as shown below by plotting the mean absorbance for each Calibrator on the y-axis versus the concentration of free PSA on the x-axis. A point-to-point curve should be drawn through the calibration points. Do not force the curve to a straight line.

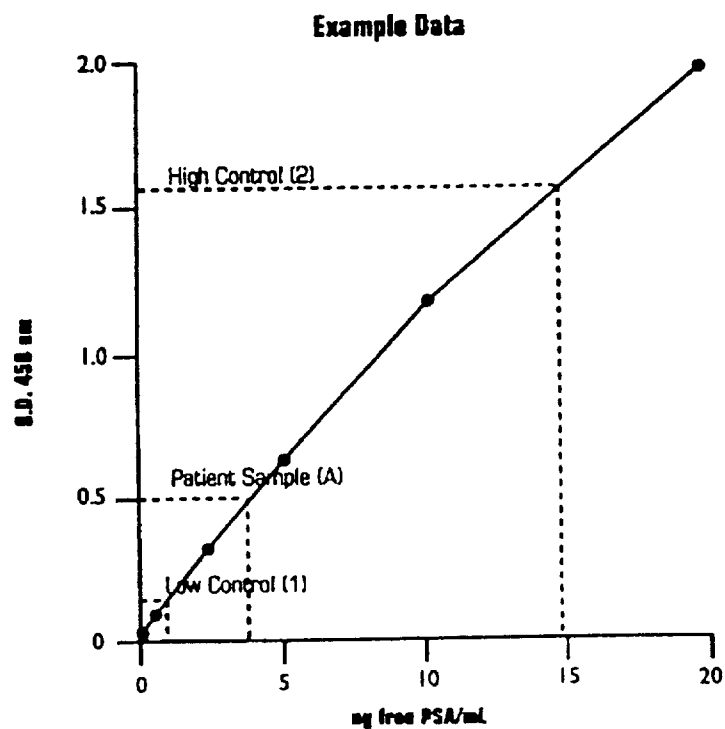
To determine the concentration of free PSA in the Controls and patient specimens, extend a horizontal line from the absorbance value for the test sample to the calibration curve. At the point of intersection of the horizontal line and the curve, drop a vertical line to the x-axis and read the concentration of free PSA in ng/mL.

If the absorbance of the sample is greater than the linear range of the microplate reader at 450 nm, the result must be reported as "greater than that value which corresponds to the linearity of the microplate reader," or the specimen must be diluted and re-assayed. The observed concentration of the diluted patient specimen must be multiplied by the dilution factor.

For further information, consult the operator's manual for the microplate reader or contact your local Sales Representative.

Example Data at 450 nm

Well #	Description	Abs. 450 nm	Mean Abs.	ng/mL
1	Zero Calibrator (A)	0.012	0.013	-----
2	" "	0.013		
3	0.5 ng/mL Calibrator (B)	0.102	0.099	-----
4	" "	0.096		
5	2 ng/mL Calibrator (C)	0.314	0.313	-----
6	" "	0.312		
7	5 ng/mL Calibrator (D)	0.771	0.761	-----
8	" "	0.750		
9	10 ng/mL Calibrator (E)	1.220	1.225	-----
10	" "	1.230		
11	20 ng/mL Calibrator (F)	2.065	2.066	-----
12	" "	2.067		
13	Low Control (1)	0.147	0.146	0.83
14	" "	0.144		
15	High Control (2)	1.641	1.637	14.90
16	" "	1.633		
17	Patient Sample (A)	0.509		3.31



### Percent free PSA

Free PSA values alone have not been shown to be effective in patient management and should not be used. Both total PSA and free PSA concentrations should be determined on the same serum specimen and used to calculate the percentage of free PSA. Percent free PSA values are then used for patient management.

$$\frac{\text{Tandem free PSA (ng/mL)}}{\text{Tandem (total) PSA (ng/mL)}} \times 100\% = \text{Percent free PSA}$$

### EXPECTED VALUES

A multi-center, prospective clinical trial was conducted to test the effectiveness of percent free PSA as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection.

All subjects were between 50 and 75 years of age, with serum PSA values between 4 and 10 ng/mL and digital rectal examination (DRE) findings that were not suspicious for cancer. These men represent the "diagnostic gray zone", in which total PSA has identified the men as high risk (25% cancer rate compared to a 4% cancer rate for the general population of men over 50 years of age), but where specificity could be improved. All men had undergone ultrasound-guided six-sector needle biopsies of the prostate, and thus had a histologically-confirmed diagnosis prior to determination of free PSA concentrations. The study was blinded; pathologists did not have access to percent free PSA values, and laboratorians did not have access to diagnoses. Exclusion criteria included acute prostatitis, urinary tract infection, prior transurethral resection of the prostate (TURP), or recent prostatic manipulation or medications that might alter serum PSA concentrations.

A total of 773 men participated in the study. Median age for both cancer and benign disease subjects was 64 years. The study population was 86% Caucasian, 9% African-American, 3% Hispanic, and 2% Asian.

Table 1 shows the expected values for free PSA (ng/mL), total PSA (ng/mL), and percent free PSA [(free PSA / total PSA) x 100%] for this population of men.

**Table 1**  
**Tandem free PSA (ng/mL), Total PSA (ng/mL) and Percent free PSA (%):**  
**Expected Values, by Diagnosis**

		<b>Benign N = 394</b>	<b>Cancer N = 379</b>	<b>Total N = 773</b>
<b>Free PSA</b>	<b>Median</b>	1.0	0.7	0.9
	<b>Mean <math>\pm</math> SD</b>	1.1 $\pm$ 0.6	0.8 $\pm$ 0.5	1.0 $\pm$ 0.6
	<b>Range</b>	0.2 - 4.9	0.2 - 3.6	0.2 - 4.9
<b>Total PSA</b>	<b>Median</b>	5.6	5.9	5.8
	<b>Mean <math>\pm</math> SD</b>	6.0 $\pm$ 1.6	6.2 $\pm$ 1.7	6.1 $\pm$ 1.6
	<b>Range</b>	4.0 - 10.0	4.0 - 10.0	4.0 - 10.0
<b>% free PSA</b>	<b>Median</b>	17.9	12.2	15.3
	<b>Mean <math>\pm</math> SD</b>	19.0 $\pm$ 7.8	13.4 $\pm$ 6.8	16.3 $\pm$ 7.9
	<b>Range</b>	4.3 - 52.2	2.3 - 42.1	2.3 - 52.2

In a prostate cancer detection program, DRE and PSA testing would identify men with non-suspicious DRE results and PSA between 4 and 10 ng/mL. Free PSA and percent free PSA would then be determined on these patients, and results would be used as an aid in patient management.

The multi-center clinical trial results demonstrated that percent free PSA may be used in two ways:

- (1) individual patient risk assessment to aid in management decisions; or
- (2) a single cutoff (men with values less than or equal to a certain cutoff would be candidates for additional follow-up procedures such as biopsy).

#### **Individual Patient Risk Assessment**

Percent free PSA may be used to determine the relative risk of prostate cancer in individual men. Family and patient history can be used in combination with percent free PSA results to determine the best individualized patient management decisions.

Table 2 shows the probability of detecting prostate cancer with needle biopsy, based on total PSA and percent free PSA results. PSA results in this table were obtained from a prior multi-center study evaluating the efficacy of total PSA for prostate cancer detection,<sup>16,17</sup> and percent free PSA results were obtained from the current study.

It can be seen that rising PSA levels increase the risk of detectable cancer. Percent free PSA can further stratify risk for men with PSA values between 4 and 10 ng/mL and non-suspicious digital rectal examination results. Lower percent free PSA values indicate higher risk. The risk of cancer ranged from 8% to 56% for this population. For purposes of comparison, the risk of prostate cancer is 4% for the general population of men over 50 years of age.<sup>16</sup>

**Table 2.**  
Probability of Prostate Cancer, Based on PSA and Percent Free PSA Results  
(for Men with Non-Suspicious DRE Results, Regardless of Patient Age)

PSA	Probability of Cancer	Percent free PSA	Probability of Cancer
0-2 ng/mL	1%	0-10%	56%
2-4 ng/mL	15%	10-15%	28%
4-10 ng/mL	25%	15-20%	20%
>10 ng/mL	>50%	20-25%	16%
		>25%	8%

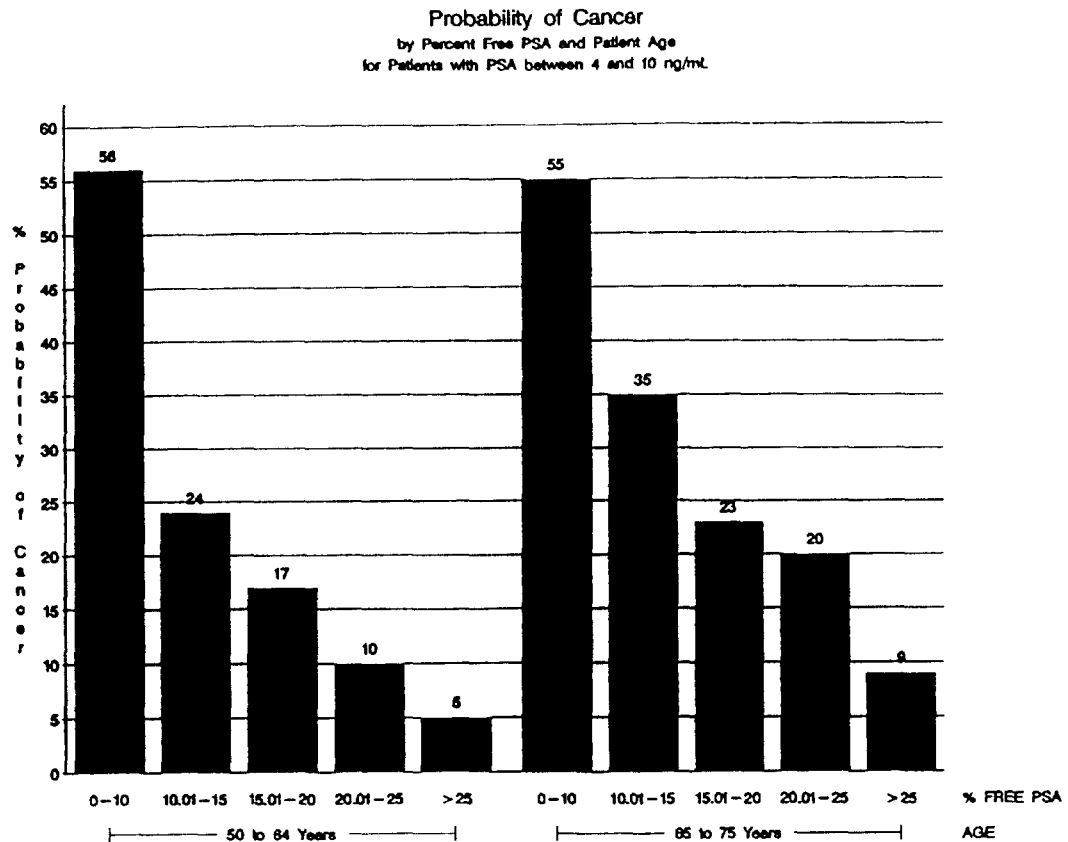
Percent free PSA values should not be interpreted as definitive evidence for the presence or absence of prostate cancer. Prostatic biopsy is required for diagnosis of cancer.

The clinical trial results also demonstrated that older men were at higher risk than younger men. The probability of cancer by percent free PSA value and age is shown in Table 3 and Figure 1.

**Table 3**  
**Probability of Prostate Cancer**  
(for Men with Non-Suspicious DRE Results  
and PSA between 4 and 10 ng/mL, by Patient Age)

Percent free PSA (%FPSA)	Patient Age	
	50 to 64 years	65 to 75 years
0.00 to 10.00%	56%	55%
10.01 to 15.00%	24%	35%
15.01 to 20.00%	17%	23%
20.01 to 25.00%	10%	20%
≥ 25.01%	5%	9%

Figure 1



### Single Cutoff

Rather than using risk assessment, a cutoff approach to patient management can also be used. Table 4 shows sensitivity (percentage of cancers detected) and specificity (percentage of biopsies avoided in men without cancer) for various percent free PSA cutoffs. A cutoff of  $\leq 25\%$  free PSA was selected based on data from the clinical trial. When men with values of 25% free PSA or less were biopsied, 95% of cancers were detected. The majority of men with PSA values between 4 and 10 ng/mL have benign disease. In this clinical trial, 20% of biopsied men with benign disease and a percent free PSA value greater than the 25% free PSA cut-off could have been spared from biopsy.

The cutoff of  $\leq 25\%$  free PSA is based on results from this clinical trial. Additional follow-up may be recommended for men with percent free PSA values above this cutoff if the physician believes it is necessary based upon other factors in the patient's medical or family history.

Table 4.  
Sensitivity and Specificity for Various Percent free PSA (%FPSA) Cutoffs  
Recommended Cutoff:  $\leq 25\%$  FPSA  
(Biopsy men with values less than or equal to this cutoff).

%FPSA Cutoffs	Sensitivity (# of cancers detected/ # of total cancers)			Specificity (# of non-cancers detected/ # of total non-cancers)		
	%	(n/N)	95% CI *	%	(n/N)	95% CI *
$\leq 25\%$	95%	(358/379)	92 - 97%	20%	(80/394)	16 - 24%
$\leq 32\%$	98%	(373/379)	96 - 99%	6%	(25/394)	4 - 9%
$\leq 55\%$	100%	(379/379)		0%	(0/394)	

\* 95% CI = 95% Confidence Intervals

Table 5 shows that the cancers occurring in men with a percent free PSA value above the 25% cutoff (i.e. those cancers which would be missed if men above the cutoff were not biopsied) are found primarily in older men with larger glands. Older men (those with less than a 10 year life expectancy) are often not affected by nor treated for prostate cancer. Thus, use of percent free PSA would result in a recommendation for biopsy in younger men, those most likely to gain from early detection.

The volume finding is clinically advantageous. Men with percent free PSA values near and above the cutoff tend to have large glands (benign prostatic hyperplasia), whereas men with cancer have lower percent free PSA values which tend to cluster progressively further away from the cutoff. Thus, when the recommendation is made not to biopsy men above the cutoff, this is the group with the lowest risk of cancer and the highest probability of benign disease (see Table 2 and discussion in previous section, "Individual Patient Risk Assessment").

**Table 5.**  
**Characteristics of Cancer Subjects Above and Below Cutoff:**  
**Patient Age and Prostate Volume**

Percent free PSA Cutoff	Median Patient Age	Median Prostate Volume
> 25% free PSA	68 years	48 cc
≤ 25% free PSA	63 years	34 cc

### **ACCEPTABILITY OF RESULTS**

- Values for duplicate determinations below 0.2 absorbance units should be within 0.02 absorbance units of the mean absorbance reading.
- Duplicate absorbance values greater than 0.2 absorbance units generally should be expected to be within 10% of the mean absorbance.
- The difference between the absorbance value of the Zero Diluent/Calibrator and the absorbance of a substrate blank should be less than 0.005 absorbance units at 450 nm. To prepare a substrate blank, add 100 µL of Substrate Reagent to an empty microplate well at the same point in time that it is added to the sample wells, then perform Steps 7-10 of the Assay Procedure.
- Recovery of the control concentrations should fall within the stated range.

### **QUALITY CONTROL**

Good laboratory practice includes the use of control specimens within an assay run to ensure that all reagents and protocols are performing properly. The Tandem-MP *free* PSA kit contains Controls which can be used to verify assay performance. The coefficient of variation for the Calibrators should be less than 10%.

### **LIMITATIONS**

Serum PSA concentrations (free, total, or percent free PSA) should not be interpreted as absolute evidence for the presence or absence of prostate cancer. Elevated total PSA concentrations or decreased percent free PSA may be observed in the serum of patients with non-malignant disorders, as well as those with prostate cancer. Furthermore, low total PSA concentrations or elevated percent free PSA are not necessarily indicative of the absence of cancer. Serum free and total PSA values should be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as digital rectal examination (DRE). Some cases of early prostate cancer will not be detected by PSA testing; the same is true for DRE. Biopsy of the prostate is the standard method used to confirm the presence or absence of prostate cancer.

The 5 alpha-reductase inhibitor drugs may affect PSA levels in some patients. Other drugs used to treat BPH may also affect PSA levels. Care should be taken in interpreting results from patients taking these drugs.

## PERFORMANCE CHARACTERISTICS

### Precision

Overall precision of the Tandem-MP *free* PSA assay was determined by duplicate analyses of 5 free PSA controls across 20 runs. The data presented were calculated based on NCCLS EP5-T2 guidelines.

Sample	Mean (ng/mL)	Within-Run (%C.V.)	Between-Run (%C.V.)	Total (%C.V.)
1	0.40	3.53	7.05	7.89
2	1.02	3.92	2.75	4.79
3	1.89	1.85	4.34	4.72
4	3.50	3.54	4.11	5.43
5	15.72	3.92	2.96	4.91

### Inter-Laboratory Precision

Inter-laboratory precision was evaluated in 3 laboratories by assaying in triplicate a panel of serum samples spanning the assay range. A table representing the results obtained with 5 of the samples is provided below.

Sample	Mean (ng/mL)	Standard Deviation	%CV
1	0.39	0.01	1.47
2	1.04	0.01	0.55
3	1.88	0.02	0.81
4	3.53	0.12	3.31
5	15.77	0.25	1.60

### Dilution

Twelve serum samples containing elevated free PSA concentrations were diluted with Zero Diluent/Calibrator and assayed in quadruplicate at multiple dilutions. Free PSA concentrations observed versus concentrations expected were analyzed by linear regression. The correlation coefficients (*r*) varied between 0.9962 and 1.000.

### Interfering Substances

- **Hemoglobin** and **bilirubin**, which were tested at concentrations up to 500 mg/dL and 20 mg/dL, respectively, do not interfere with the Tandem-MP *free* PSA Assay.
- **Triglycerides**, which were tested at concentrations up to 2500 mg/dL, do not interfere with the Tandem-MP *free* PSA Assay.
- When **total protein** was tested at concentrations between 4 and 17 g/dL, no significant effect on the results was observed.
- Cross-reactivity with PSA-ACT was determined to be less than 1%.

### Commonly Used Drugs

Various concentrations of drugs were added to serum samples containing free PSA and assayed in replicates of 4. The drugs and the highest concentrations tested are:

acetaminophen	0.2 mg/mL
aspirin	0.5 mg/mL
ibuprofen	0.4 mg/mL
naproxin sodium	1.0 mg/mL
hydrocodone	240 ng/mL
cimetidine	0.1 mg/mL
capotril	4.0 µg/mL
lovastatin	270 ng/mL
nifedipine	270 ng/mL
metoprolol	2.7 µg/mL
finasteride	370 ng/mL
terazosin hydrochloride	1.45 mg/mL
ciprofloxacin	46 µg/mL
sulfamethoxazole	116.5 µg/mL (in combination with)
trimethoprim	9.7 µg/mL
doxycycline	2.6 µg/mL
clomipramine HCl	2.7 µg/mL
fluoxetine	0.55 µg/mL
furosemide	20 µg/mL
methotrexate	13.2 µg/mL
prednisone	1.65 µg/mL
tavist-1	2.7 µg/mL

At the concentrations listed above, these drugs did not interfere with the recovery of free PSA from the serum samples.

### Minimum Detectable Concentration

The minimum detectable concentration of Tandem-MP *free* PSA is less than 0.05 ng/mL. The minimum detectable concentration is defined as that concentration of free PSA that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 22 replicate determinations of the Zero Diluent/Calibrator (A).

### Trouble-Shooting Guide

Occasionally one may encounter poor reproducibility or high absorbance for the Zero Diluent/Calibrator; possible causes and solutions are provided below.

Cause: Insufficient washing of the microplate wells.

Solution: Ensure proper washing technique. (See Procedural Notes #3).

Cause: Contamination of the Calibrators, Controls and/or patient specimens

Solution: Ensure that proper pipetting techniques with minimal carryover are employed.

Cause: Inaccurate rotation speed

Solution: Rotate plates at a speed such that the fluid in each well clearly forms a vortex but does not rise above the upper edge of the wells.

Cause: Scratches or fingerprints on the bottom of the wells, lint or debris on the inside of the wells

Solution: Measure and subtract absorbances at a reference wavelength (600-650 nm) or wipe the underside of the plate.

Cause: Inconsistent pipetting times

Solution: Ensure that the elapsed time for pipetting reagents from beginning to end (without interruption) is the same  $\pm 2$  minutes for both the Substrate Reagent addition step and the Quench Reagent addition step.

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Manufactured by: Hybritech Incorporated San Diego, CA 92121 U.S.A.

For additional information, please contact Hybritech Technical Support:

U.S.A. : 1-800-854-1957  
EUROPE : 32 4 361 6363

HYBRITECH INCORPORATED, San Diego, CA 92121 U.S.A.  
HYBRITECH EUROPE S.A. B - 4031 Liege, Belgium

Hybritech Incorporated, February 1998

# Hybritech's Tandem<sup>®</sup> *free* PSA Assay

## Information For Physicians

*Provided as a service of Hybritech Incorporated*

## FOREWORD

This publication is intended to provide the physician with supplementary information regarding the use of free PSA testing as an aid in prostate cancer detection. Please call Hybritech Technical Support at 1-800-854-1957 to obtain additional copies of this brochure.

Hybritech Incorporated, P.O. Box 269006, San Diego, CA 92196-9006

**CAUTION:** *Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by or on the order of a physician.*

### WARNING

**The Tandem free PSA Assay should be used only with the Tandem (total) PSA Assay to calculate the ratio of free PSA to total PSA (percent free PSA). Use of another manufacturer's total PSA assay may result in:**

**(1) an inappropriate population of patients selected for free PSA testing; and**

**(2) significantly different percent free PSA values, cutoffs and cancer probabilities than presented in the Clinical Studies section of this brochure.**

**Results contained in this brochure apply only to percent free PSA as measured by the Tandem free PSA and (total) PSA Assays.**

**The concentration of free PSA and total PSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must specify the manufacturer of the free and total PSA assays used. Values obtained with different manufacturers' assays cannot be used interchangeably.**

**Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by or on the order of a physician.**

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## INTRODUCTION

Prostate cancer is the most common type of cancer found in men in the United States, with an incidence of approximately one case for every ten men. It is also the second leading cause of cancer deaths among American men.<sup>1</sup>

Prostate-specific antigen (PSA) was identified and purified by Wang and co-workers in 1979.<sup>2</sup> PSA, a serine protease, is produced by the epithelial cells of the prostate, and is produced by both benign and malignant cells. Abnormalities in the prostate gland architecture resulting from trauma or disease can lead to "leakage" of PSA into the bloodstream.

A multi-center, prospective clinical trial conducted by Hybritech found that Tandem<sup>®</sup> PSA was a safe and effective aid in the detection of prostate cancer.<sup>3</sup> In this study of 6,630 men, PSA and DRE performed together detected approximately 81% more prostate cancer than DRE alone.

However, PSA is a tissue-specific rather than tumor-specific protein that has been shown to be elevated in both benign prostatic conditions and prostate cancer. Although measurement of PSA has been shown to be valuable in the early detection of prostate cancer, it is limited by its relative lack of specificity when the PSA concentration is in the range of 4 to 10 ng/mL. The false positive rate of the test in this range is reported to be 75%. It should be noted, however, that mammography, a widely-accepted cancer screening method, has a false positive rate of 80%. Serum total PSA is effective in the detection of prostate cancer, but any method that would increase specificity would be of great benefit to the medical community.

The American Urological Association and the American Cancer Society have recommended that men over the age of 50 have an annual prostate evaluation consisting of a digital rectal examination (DRE) and a serum PSA test. Men in high risk groups and those with a family history of prostate cancer are urged to begin prostate examinations at age 40.

PSA exists primarily as three forms in serum.<sup>4</sup> One form of PSA is believed to be enveloped by a protease inhibitor, alpha-2 macroglobulin,<sup>5</sup> and has been shown to lack immunoreactivity. A second form is complexed to another protease inhibitor, alpha-1 antichymotrypsin (ACT).<sup>5,6</sup> The third form of PSA is not complexed to a protease inhibitor, and is termed free PSA.<sup>5,6</sup> The latter two forms are immunologically detectable in commercially available PSA assays and are referred to collectively as total PSA.

Previous reports have shown that measurement of PSA forms enhances the specificity of total PSA for prostate cancer detection and is useful in the differentiation of prostate cancer from benign prostatic conditions.<sup>7,8</sup> In patients with elevated PSA concentrations, men with prostate cancer tend to have lower percent free PSA (free PSA/total PSA) values than men with benign disease.<sup>9-12</sup> This difference in the distribution of percent free PSA values in men with and without cancer may be used to select cutoffs for biopsy decisions, maintaining 90% to 95% sensitivity, while sparing 20% to 30% of men with benign disease from biopsy.

Percent free PSA may also be used for risk assessment, to determine the probability of cancer for an individual patient. Lower percent free PSA values are associated with higher risk of cancer.<sup>9-12</sup>

In current clinical practice, prostate biopsy is recommended for men with a suspicious DRE result, regardless of the PSA level (Table 1). Patients with PSA greater than 10 ng/mL also undergo biopsy since the rate of cancer is high in this group (>50%). Biopsy is recommended for men with non-suspicious DRE results and PSA between 4 and 10 ng/mL, and 25% of these men have cancer. But specificity could be improved in this population since 75% of the biopsies are negative. Therefore, this is the group selected for Hybritech's multi-center percent free PSA clinical trial.

**Table 1. Current Clinical Practice (Biopsy/No Biopsy) and Proportion of Men with Cancer, Based on Tandem PSA and DRE Findings.**

	Tandem PSA ng/mL			
	0 - 2	2-4	4-10	>10
DRE -	No Biopsy (1% Cancer)	No Biopsy (15% Cancer)	Biopsy (25% Cancer)	Biopsy (>50% Cancer)
DRE +	Biopsy (5% Cancer)	Biopsy (20% Cancer)	Biopsy (45% Cancer)	Biopsy (>75% Cancer)

Gray Box: Percent free PSA Clinical Trial Population  
(Comparison: Mammography for breast cancer detection = 20% cancer)

This trial was designed to evaluate the ability of percent free PSA as measured by Hybritech's Tandem assays to distinguish prostate cancer from benign prostatic conditions in men aged 50 years or older with a PSA between 4 and 10 ng/mL and a non-suspicious DRE. This study found that use of percent free PSA would have eliminated 20% of the unnecessary biopsies in men without cancer, while still detecting 95% of the cancers.<sup>13</sup>

The results of this study are summarized in this brochure. This information is designed to instruct physicians on the use of Tandem free PSA and provide them with a valuable tool to enhance their cancer detection and patient management strategies.

In the previously mentioned Hybritech-sponsored clinical study of the Tandem (total) PSA assay for prostate cancer detection, 9% of the 6,630 men tested had a non-suspicious DRE and a PSA between 4 and 10 ng/mL.<sup>3</sup> Thus, in a prostate cancer detection program, DRE and PSA testing would identify this subgroup of all men undergoing evaluation for prostate cancer. Free PSA and percent free PSA (ratio of free PSA to total PSA x 100) would then be determined for these patients, and results would be used as an aid in patient management.

## INTENDED USE

The Tandem *free* PSA Assays are *in vitro* devices for the quantitative measurement of free prostate specific antigen (free PSA) in human serum.

Hybritech's Tandem *free* PSA assays are intended to be used with Tandem (total) PSA to calculate the ratio of free PSA to total PSA expressed as a percentage (percent free PSA).

Percent free PSA as measured by Hybritech's Tandem assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection in men aged 50 years or older who have total PSA values between 4 and 10 ng/mL and digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer.

## SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is necessary. Specimens for free PSA testing should be drawn prior to such prostatic manipulations as digital rectal examination (DRE), prostatic massage, transrectal ultrasound (TRUS), and prostatic biopsy. DRE may cause a transient increase in both free and total PSA.<sup>14</sup> A repeat total PSA measurement in the case of borderline elevation is recommended.<sup>15</sup>

Transrectal needle biopsy has also been shown to cause transient increases in free PSA and persisting total PSA elevations,<sup>14,15</sup> thus a six-week waiting period between needle biopsy and PSA sampling has been recommended.

Serum is required for the Tandem *free* PSA Assay. Plasma samples should **not** be used.

Only blood drawn by an acceptable medical technique into a collection tube with no anticoagulants should be used. Specimens should be collected in such a way as to avoid hemolysis.

The specimen should be allowed to clot and the serum separated by centrifugation. **Specimens should be processed (centrifuged) and refrigerated within 3 hours of blood draw.**

If the serum sample is to be assayed within 24 hours after collection, the specimen should be stored in a refrigerator at 2°C to 8°C. Specimens held for longer times (up to 5 months) should be frozen at -20°C or colder.<sup>16,17</sup> Specimens to be held for longer than 5 months should be frozen at -70°C.<sup>16,17</sup> Repeated freeze-thaw cycles have no effect on free PSA, total PSA, or percent free PSA.<sup>16</sup> However, prompt refreezing of the thawed samples is recommended.

Turbid serum samples or samples containing particulate matter should be centrifuged prior to assay.

## CLINICAL STUDY RESULTS

A multi-center, prospective clinical trial was conducted to test the effectiveness of percent free PSA as an aid in distinguishing prostate cancer from benign prostatic conditions, when used in conjunction with Tandem (total) PSA for prostate cancer detection.

All subjects were between 50 and 75 years of age, with serum PSA values between 4 and 10 ng/mL and digital rectal examination (DRE) findings that were not suspicious for cancer. These men represent the "diagnostic gray zone", in which total PSA has identified the men as high risk (25% cancer rate compared to a 4% cancer rate for the general population of men over 50 years of age), but where specificity could be improved. All men had undergone ultrasound-guided six-sector needle biopsies of the prostate, and thus had a histologically-confirmed diagnosis prior to determination of free PSA concentrations. The study was blinded; pathologists did not have access to percent free PSA values, and laboratorians did not have access to diagnoses. Exclusion criteria included acute prostatitis, urinary tract infection, prior transurethral resection of the prostate (TURP), or recent prostatic manipulation or medications that might alter serum PSA concentrations.

A total of 773 men participated in the study. Median age for both cancer and benign disease subjects was 64 years. The study population was 86% Caucasian, 9% African-American, 3% Hispanic, and 2% Asian.

Table 2 shows the expected values for free PSA (ng/mL), total PSA (ng/mL), and percent free PSA [(free PSA / total PSA) x 100%] for this population of men.

**Table 2**  
**Tandem free PSA (ng/mL), Total PSA (ng/mL) and Percent free PSA (%):**  
**Expected Values, by Diagnosis**

		<b>Benign N = 394</b>	<b>Cancer N = 379</b>	<b>Total N = 773</b>
<b>Free PSA</b>	<b>Median Mean <math>\pm</math> SD Range</b>	1.0 1.1 $\pm$ 0.6 0.2 - 4.9	0.7 0.8 $\pm$ 0.5 0.2 - 3.6	0.9 1.0 $\pm$ 0.6 0.2 - 4.9
<b>Total PSA</b>	<b>Median Mean <math>\pm</math> SD Range</b>	5.6 6.0 $\pm$ 1.6 4.0 - 10.0	5.9 6.2 $\pm$ 1.7 4.0 - 10.0	5.8 6.1 $\pm$ 1.6 4.0 - 10.0
<b>% free PSA</b>	<b>Median Mean <math>\pm</math> SD Range</b>	17.9 19.0 $\pm$ 7.8 4.3 - 52.2	12.2 13.4 $\pm$ 6.8 2.3 - 42.1	15.3 16.3 $\pm$ 7.9 2.3 - 52.2

In a prostate cancer detection program, DRE and PSA testing would identify men with non-suspicious DRE results and PSA between 4 and 10 ng/mL. Free PSA and percent free PSA would then be determined on these patients, and results would be used as an aid in patient management.

The multi-center clinical trial results demonstrated that percent free PSA may be used in two ways:

- (1) individual patient risk assessment to aid in management decisions; or
- (2) a single cutoff (men with values less than or equal to a certain cutoff would be candidates for additional follow-up procedures such as biopsy).

### INDIVIDUAL PATIENT RISK ASSESSMENT

Percent free PSA may be used to determine the relative risk of prostate cancer in individual men. Family and patient history can be used in combination with percent free PSA results to determine the best individualized patient management decisions.

Table 3 shows the probability of detecting prostate cancer with needle biopsy, based on total PSA and percent free PSA results. PSA results in this table were obtained from a prior multi-center study evaluating the efficacy of total PSA for prostate cancer detection,<sup>3,18</sup> and percent free PSA results were obtained from the current study.

It can be seen that rising PSA levels increase the risk of detectable cancer. Percent free PSA can further stratify risk for men with PSA values between 4 and 10 ng/mL and non-suspicious digital rectal examination results. Lower percent free PSA values indicate higher risk. The risk of cancer ranged from 8% to 56% for this population. For purposes of comparison, the risk of prostate cancer is 4% for the general population of men over 50 years of age.<sup>3</sup>

**Table 3.**

Probability of Prostate Cancer, Based on PSA and Percent Free PSA Results  
(for Men with Non-Suspicious DRE Results, Regardless of Patient Age)

PSA	Probability of Cancer	Percent free PSA	Probability of Cancer
0-2 ng/mL	1%	0-10%	56%
2-4 ng/mL	15%	10-15%	28%
4-10 ng/mL	25%	15-20%	20%
>10 ng/mL	>50%	20-25%	16%
		>25%	8%

Percent free PSA values should not be interpreted as definitive evidence for the presence or absence of prostate cancer. Prostatic biopsy is required for diagnosis of cancer. However 20% of cancers are missed on the first biopsy. A patient who has undergone one biopsy with negative findings may be advised to undergo a second biopsy if the percent free PSA value indicates high risk.

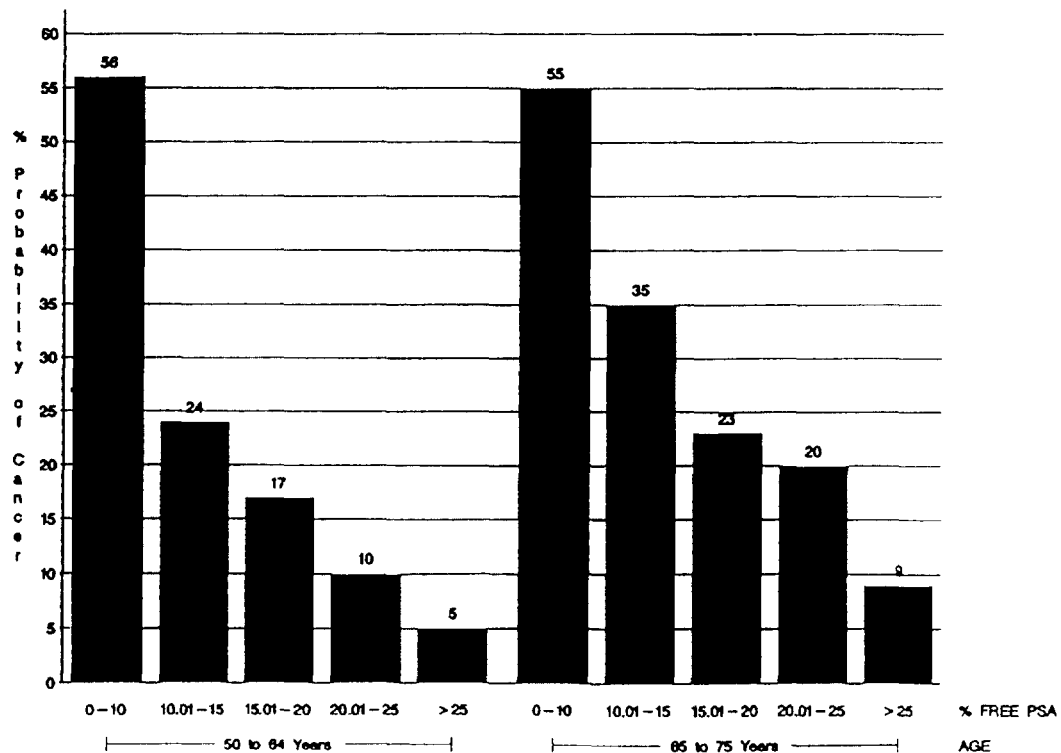
The clinical trial results also demonstrated that older men were at higher risk than younger men. The probability of cancer by percent free PSA value and age is shown in Table 4 and Figure 1.

Table 4  
Probability of Prostate Cancer  
(for Men with Non-Suspicious DRE Results  
and PSA between 4 and 10 ng/mL, by Patient Age)

Percent free PSA (%FPSA)	Patient Age	
	50 to 64 years	65 to 75 years
0.00 to 10.00%	56%	55%
10.01 to 15.00%	24%	35%
15.01 to 20.00%	17%	23%
20.01 to 25.00%	10%	20%
<u>≥ 25.01%</u>	5%	9%

Figure 1

Probability of Cancer  
by Percent Free PSA and Patient Age  
for Patients with PSA between 4 and 10 ng/mL



## SINGLE CUTOFF

Rather than using risk assessment, a cutoff approach to patient management can also be used for interpreting patient results. Table 5 shows sensitivity (percentage of cancers detected) and specificity (percentage of biopsies avoided in men without cancer) for various percent free PSA cutoffs. A cutoff of  $\leq 25\%$  free PSA was selected based on data from the clinical trial. When men with values of 25% free PSA or less were biopsied, 95% of cancers were detected. The majority of men with PSA values between 4 and 10 ng/mL have benign disease. In this clinical trial, 20% of biopsied men with benign disease and a percent free PSA value greater than the 25% free PSA cut-off could have been spared from biopsy.

The cutoff of  $\leq 25\%$  free PSA is based on results from this clinical trial. Additional follow-up may be recommended for men with percent free PSA values above this cutoff if the physician believes it is necessary based upon other factors in the patient's medical or family history.

**Table 5.**  
**Sensitivity and Specificity for Various Percent free PSA (%FPSA) Cutoffs**  
**Recommended Cutoff:  $\leq 25\%$  FPSA**  
**(Biopsy men with values less than or equal to this cutoff).**

%FPSA Cutoffs	Sensitivity (# of cancers detected/ # of total cancers)			Specificity (# of non-cancers detected/ # of total non-cancers)		
	%	(n/N)	95% CI *	%	(n/N)	95% CI *
$\leq 25\%$	95%	(358/379)	92 - 97%	20%	(80/394)	16 - 24%
$\leq 32\%$	98%	(373/379)	96 - 99%	6%	(25/394)	4 - 9%
$\leq 55\%$	100%	(379/379)		0%	(0/394)	

\* 95% CI = 95% Confidence Intervals

Table 6 shows that the cancers occurring in men with a percent free PSA value above the 25% cutoff (i.e. those cancers which would be missed if men above the cutoff were not biopsied) are found primarily in older men with larger glands. Older men (those with less than a 10 year life expectancy) are often not affected by nor treated for prostate cancer. Thus, use of percent free PSA would result in a recommendation for biopsy in younger men, those most likely to gain from early detection.

The volume finding is clinically advantageous. Men with percent free PSA values near and above the cutoff tend to have large glands (benign prostatic hyperplasia), whereas men with cancer have lower percent free PSA values which tend to cluster progressively further away from the cutoff. Thus, when the recommendation is made not to biopsy men above the cutoff, this is the group with the lowest risk of cancer and the highest probability of benign disease (see Table 3 and discussion in the previous section, "Individual Patient Risk Assessment").

**Table 6.**  
**Characteristics of Cancer Subjects Above and Below Cutoff:**  
**Patient Age and Prostate Volume**

Percent free PSA Cutoff	Median Patient Age	Median Prostate Volume
> 25% free PSA	68 years	48 cc
≤ 25% free PSA	63 years	34 cc

Thus the study found that the cancers above the cutoff, which would be missed, were more prevalent in older men. These cancers were also more likely to be less aggressive. Prostate-cancer is generally slow-growing. Therefore monitoring these cancer patients over time could allow for future follow-up if total PSA increases or percent free PSA decreases.

## **LIMITATIONS**

- **General Information**

Serum PSA concentrations (free, total, or percent free PSA) should not be interpreted as absolute evidence for the presence or absence of prostate cancer. Elevated total PSA concentrations or decreased percent free PSA may be observed in the serum of patients with non-malignant disorders, as well as those with prostate cancer. Furthermore, low total PSA concentrations or elevated percent free PSA are not necessarily indicative of the absence of cancer. Serum free and total PSA values should be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as digital rectal examination (DRE). Some cases of early prostate cancer will not be detected by PSA testing; the same is true for DRE. Biopsy of the prostate is the standard method used to confirm the presence or absence of prostate cancer.

The 5 alpha-reductase inhibitor drugs may affect PSA levels in some patients. Other drugs used to treat BPH may also affect PSA levels. Care should be taken in interpreting results from patients taking these drugs.

- Appropriate Use of Free PSA Assay Results

Study results were obtained using Hybritech's Tandem free PSA and total PSA assays. Recent studies have shown that percent free PSA cutoffs and clinical performance differ when various combinations of free and total PSA assays from different manufacturers are used.<sup>19-22</sup> Mean percent free PSA values from identical serum samples may be two-fold higher using different assay combinations<sup>20</sup> and 95% sensitivity cutoffs may vary from 22% to 34% using different assay combinations.<sup>22</sup> Thus, the study results presented in this brochure apply only to the assays manufactured by Hybritech; results from other manufacturers may vary.

The efficacy of a total PSA assay for cancer detection should be proven prior to adding a second marker (percent free PSA) to enhance this indication. Each total PSA assay should establish its own reference range, positive predictive values, sensitivity and specificity. Numerous total PSA assays are available; however they are not all equimolar and do not detect various PSA forms equally. These assays are also not calibrated to one another or to one set of standards. Therefore, the potential for incorrect percent free PSA values is high if a free PSA assay from one manufacturer is used with a total PSA assay from another manufacturer. In addition, use of another manufacturer's total PSA assay which is not calibrated to the Tandem (total) PSA assay in the 4 to 10 ng/mL range could result in the wrong patient population being sent on for percent free PSA testing. The 4 to 10 ng/mL range for the Tandem assay could be 2.5 to 8 ng/mL for another manufacturer's total PSA assay. Also, patients with benign disease and high free PSA levels could be flagged by a non-equimolar assay to proceed to percent free PSA testing (non-equimolar assays over-report free PSA), whereas cancer patients may fall below the 4 ng/mL cutoff and remain untested and unbiopsied.

The use of free and total PSA assays to generate a percent free PSA value must be performed in a way that ensures accurate, reliable and easily interpretable results. To obtain this accuracy and reliability, the free and total PSA assays used to generate results must be from the same manufacturer. Physicians can ensure this by requesting that laboratories provide them with the name of the PSA assays used.

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